VOL. IX JANUARY, 1920 No. 1

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

CHEMICAL LIBRARY
CENTRAL LIBRARY
APR 26 1920
UNIV. OF MICH.

JACOB G. LIPMAN, Editor-in-Chief

CARL R. WOODWARD
INGRID C. NELSON } Assistant Editors

IN CONSULTATION WITH

DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn.
PROF. C. BARTHEL
Centralanstalten för Försöksväsendet på Jordbruksområdet, Stockholm, Sweden
DR. M. W. BEIJERINCK
Technische-Hoogeschool, Delft, Holland
PROF. A. W. BLAIR
Rutgers College, New Brunswick, N. J.
DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa
ALBERT BRUNO
Ministry of Agriculture, Paris, France
DIRECTOR H. R. CHRISTENSEN
Statens Planteavls-Laboratorium, Copenhagen, Denmark
DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y.
DR. H. VON FEILITZEN
Svenska Mosskulturföreningen, Jönköping, Sweden
DR. E. B. FRED
University of Wisconsin, Madison, Wis.
DR. J. E. GREAVES
Utah Agricultural College, Logan, Utah
DIRECTOR A. TH. GREGOIRE
Agricultural Experiment Station, Gembloux, Belgium
DR. R. GREIG-SMITH
Linnean Society, Sydney, New South Wales
DR. B. L. HARTWELL
Rhode Island Experimental Station, Kingston, R. I.
DR. C. B. LIPMAN
University of California, Berkeley, Calif.
DR. BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.
DR. F. LÖHNIS
U. S. Department of Agriculture, Washington, D. C.
DR. T. L. LYON
Cornell University, Ithaca, N. Y.
DR. M. M. MCCOOL
Michigan Agricultural College, East Lansing, Mich.
DR. W. H. MCINTIRE
Tennessee Experiment Station, Knoxville, Tenn.
PROF. C. A. MOOERS
Tennessee Experiment Station, Knoxville, Tenn.
PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy
DR. E. J. RUSSELL
Rothamsted Experiment Station, Harpenden, England
DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.
PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio

PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

Copyright 1919, by Williams & Wilkins Company

Price { $6.00 per year, two volumes, United States, Mexico, Cuba
$6.25 per year, two volumes, Canada
$6.50 per year, two volumes, other countries

SOIL SCIENCE

Contents for January, 1920

C. R. Fellers and F. E. Allison. The Protozoan Fauna of the Soils of New Jersey 1

L. P. Howard. The Reaction of the Soil as Influenced by the Decomposition of Green Manures 27

C. T. Hirst and J. E. Greaves. Some Factors Influencing the Quantitative Determination of Chlorides in Soil 41

T. L. Lyon, J. A. Bizzell and B. D. Wilson. The Formation of Nitrates in a Soil Following the Growth of Red Clover and of Timothy 53

Firman E. Bear and George M. McClure. Sampling Soil Plots 65

Seth S. Walker. The Effect of Aeration and Other Factors on the Lime Requirement of a Muck Soil 77

HENRY HEIL CHEMICAL CO.

210-214 South 4th Street

ST. LOUIS, - MISSOURI

Importers and Manufacturers of

CHEMICAL APPARATUS, CHEMICALS

Assay and Laboratory Supplies Generally

We have the stock and can make prompt delivery

SPECIALTIES:

Kavalier Bohemian Normal Glassware; Best Grade of American Glassware, "Fry", "Non-Sol", "Perfection" and "Pyrex"; Max Dreverhoff, Schleicher & Schuell, Swedish and English Filtering Papers; Royal Berlin and Haldenwanger Berlin Porcelain Ware; Best American Porcelain Ware; Troemner & Becker Balances and Weights; Platinum Wire, Foil and Ware; Fused Silica Laboratory Utensils "Vitreosil"; Hanovia Pure Transparent Quartz Glass; Alundum Ware; Hoskins' Electric Furnaces; Le Chatelier's Pyrometers; Bausch & Lomb Microscopes and Accessories; Hydrometers and Thermometers of every description; Chemically Pure Acids and Ammonia, Molybdic Acid and Molybdate of Ammonia; Merck's, Baker & Adamson's and J. T. Baker Chemical Co.'s Chemicals and Reagents; Caustic Potash and Soda, purified in sticks and pure by alcohol; Acetate of Lead, Cyanide Mixture, Soda Ash and Commercial Caustic Soda

CONTENTS

	Page
The Protozoan Fauna of the Soils of New Jersey. C. R. Fellers and F. E. Allison.	1
The Reaction of the Soil as Influenced by the Decomposition of Green Manures. L. P. Howard	27
Some Factors Influencing the Quantitative Determination of Chlorides in Soil. C. T. Hirst and J. E. Greaves	41
The Formation of Nitrates in a Soil Following the Growth of Red Clover and of Timothy. T. L. Lyon, J. A. Bizzell and B. D. Wilson	53
Sampling Soil Plots. Firman E. Bear and George M. McClure	65
The Effect of Aeration and Other Factors on the Lime Requirement of a Muck Soil. Seth S. Walker	77
The Lime Factor in Permanent Soil Improvement: I. Rotations without Legumes. J. G. Lipman and A. W. Blair	83
The Lime Factor in Permanent Soil Improvement. II. Rotations with Legumes. J. G. Lipman and A. W. Blair	91
Colorimetric Determination of Hydrogen-ion Concentration without Buffer Mixtures, with Especial Reference to Soils. Louis J. Gillespie	115
The Comparative Rate of Decomposition of Green and Cured Clover Tops in Soil. Albert L. Whiting and Warren R. Schoonover	137
Concerning the Effect of Heat on the Reaction between Lime-Water and Acid Soils. R. H. Robinson	151
The Moisture Equivalent in Relation to the Mechanical Analysis of Soils. Howard E. Middleton	159
The Influence of Sand upon the Concentration and Reaction of a Nutrient Solution for Plants. John W. Shive	169
Experiments on the Value of Common Rock Salt and Sulfur for Killing Live Stumps. W. Rudolfs	181
A New Soil Elutriator. Willard Gardner	191
Reduction Potentials of Bacterial Cultures and of Water-Logged Soils. Louis J. Gillespie	199
Further Studies on the Freezing-Point Lowering of Soils and Plants. M. M. McCool and C. E. Millar	217
The Effect of Leaching on the Availability of Rock Phosphate to Corn. F. C. Bauer.	235
The Lime Requirement of Soils According to the Veitch Method, Compared with the Hydrogen-ion Concentration of the Soil Extract. A. W. Blair and A. L. Prince.	253
Hydrogen-ion Concentration Measurements of Soils in Connection with their "Lime-Requirements." Jacob S. Joffe	261
The Relative Absorption by Soil of Sodium Carbonate and Sodium Chloride. Thomas H. Kearney	267
Symbiotic Nitrogen Fixation as Influenced by the Nitrogen in the Soil, William Albert Albrecht	275
The Potential Biochemical Activity of the Spores of Soil Bacteria. J. R. Neller	329
Studies on the Reaction of Plant Juices. A. R. C. Haas	341
Field Experiments on the Availability of Nitrogenous Fertilizers, 1908–1917. J. G. Lipman and A. W. Blair	371

369383

Page

The Relation of Sulfur to Soil Acidity and to the Control of Potato Scab. WILLIAM H. MARTIN 393

Effect of Various Soluble Salts and Lime on Evaporation, Capillary Rise, and Distribution of Water in Some Agricultural Soils. M. I. WOLKOFF 409

The Effect of Gypsum on Bacterial Activities in Soils. T. M. SINGH 437

The Water-Supplying Power of the Soil as Related to the Wilting of Plants. BURTON E. LIVINGSTON AND RIICHIRO KOKETSU 469

Carriers of Nitrogen in Fertilizers. CHARLES E. THORNE 487

ILLUSTRATIONS

PLATES

The Protozoan Fauna of the Soils of New Jersey

Page

Plate 1. Rhizopoda and Heliozoa 19
Plate 2. Mastigophora (Flagellates) 21
Plate 3. Ciliata 23
Plate 4. Ciliata (continued), soil algae and diatoms 25

Experiments on the Value of Common Rock Salt and Sulfur for Killing Live Stumps

Plate 1. Fig. 1. Cleared land in early spring when the applications were made. Fig. 2. Same Field in September 187
Plate 2. Fig. 1. Plot which received approximately 2 tons of salt per acre, scattered broadcast. Fig. 2. Check plots 189

A New Soil Elutriator

Plate 1. Microphotographs of soil particles from the respective units of the elutriator after a first, second and third elutriation 197

Further Studies on the Freezing-Point Lowering of Soils and Plants

Plate 1. Fig. 1. Plant house closed. Fig. 2. Plant house closed 229
Plate 2. Plant House Open 231
Plate 3. Fig. 1. Corn: Period of growth 30 days. Fig. 2. Corn: Period of growth 60 days 233

The Effect of Leaching on the Availability of Rock Phosphate to Corn

Plate 1. The effect of leaching and of the form of phosphate and nitrogen salt on the growth of corn 249
Plate 2. The effect of leaching on the availability of rock phosphate to corn 251

Symbiotic Nitrogen Fixation as Influenced by the Nitrogen in the Soil

Plate 1. Soybeans on soil treated with nitrates, clover tops, and with clover tops and nitrates 321
Plate 2. Cowpeas, at 50 days, on soil treated with nitrates and cowpeas as the second crop on soil treated with clover tops 323
Plate 3. Cowpeas, at 75 days, on soil treated with nitrates and cowpeas as the second crop on soil treated with clover tops 325
Plate 4. Cowpeas on soil treated with nitrates and cowpeas as the third crop on soil treated with clover tops 327

Studies on the Reaction of Plant Juices

Plate 1. Effect on the growth of white mustard plants of the addition of lime to Plainfield sand 369

TEXT FIGURES

Sampling Soil Plots

Page
Fig. 1. Location of borings over plot 68
2. Nitrogen content of soil and subsoil samples chosen from Plot 1 71
3. Nitrogen content of soil and subsoil samples chosen from plot 2 72
4. Proposed arrangement of borings in future sampling 73

Colorimetric Determination of Hydrogen-Ion Concentration Without Buffer Mixtures, With Especial Reference to Soils

Fig. 1. Comparator for hydrogen-ion exponent measurements 119

The Moisture Equivalent in Relation to the Mechanical Analysis of Soils

Fig. 1. Logarithmic chart for the calculation of moisture equivalent 165

A New Soil Elutriator

Fig. 1. Diagrammatic sketch of elutriator showing complete series of nineteen units, together with feeding and stirring device 193
2. Plot of the grams-diameter distribution function from actual count and measurement with the aid of the microscope 193

Reduction Potentials of Bacterial Cultures and of Water-Logged Soils

Fig. 1. Reduction potentials of bacterial cultures 205
2. "Inverted-vial electrode" 206
3. Reduction potentials of bacterial suspensions 208
4. Reduction potentials of water-logged soils at various times 210

The Effect of Leaching on the Availability of Rock Phosphate to Corn

Fig. 1. Effect of leaching on the weight of dry matter, and content of phosphorus, calcium and nitrogen of corn grown with the treatments indicated 238
2. Influence of NH_4NO_3 compared with $NaNO_3$ on the leaching of calcium bicarbonate from rock phosphate in corn cultures 242

The Lime Requirements of Soils According to the Veitch Method, Compared with the Hydrogen-ion Concentration of the Soil Extract

Fig. 1. Diagram of pH values 256
2. Diagram of lime requirements according to the Veitch method 257

Hydrogen-ion Concentration Measurements of Soils in Connection with Their "Lime-Requirements"

Fig. 1. Curves of hydrogen-ion concentration of samples 14, 21, 22, 23 and 24 264
2. Curves of hydrogen-ion concentration of samples 13, 15, 18, and 20 265

The Relative Absorption by Soil of Sodium Carbonate and Sodium Chloride

Fig. 1. Ratio of resistance of sand plus 20 cc. of solution to that of 20 cc. of free solution 271

Symbiotic Nitrogen Fixation as Influenced by the Nitrogen in the Soil

Fig. 1. Graphs showing nitrogen fixation by soybeans on soils treated with nitrates, clover tops, and with nitrates and clover tops 296

Page

2. Graphs showing nitrogen fixation by cowpeas on soils treated with nitrates, and with clover tops 300
3. Graphs showing nitrogen fixation by cowpeas on soils treated with nitrates, and with clover tops 308

THE POTENTIAL BIOCHEMICAL ACTIVITY OF THE SPORES OF SOIL BACTERIA

Fig. 1. Curves showing the relation between the increase in depth of soil and the decrease in bacterial numbers which survived heating 331

STUDIES ON THE REACTION OF PLANT JUICES

Fig. 1. The small hydrogen electrode apparatus drawn to scale 343
2. Curves showing the actual and total acidities of the juices of plants grown on limed and unlimed soil 348
3. Curves showing the actual and total acidities of the juice of barley, oat, and corn plants grown on limed and unlimed soil 349
4. Curves showing the actual and total acidities of leaves, stems, petioles, and roots of medium-red clover plants grown with and without liming 350
5. Curves showing the actual and total acidities of the juice of buckwheat tops and of the juices of alfalfa tops, lupine tops, and lupine roots grown with and without liming 353
6. Curves showing the actual and total acidities of the juice of the leaves and stems of orchard grass 354
7. Curves showing the acid reserve of tops of the second year's growth of sweet clover and of medium-red clover cut on the same day, and that of the roots of both species obtained 3 days later 360
8. Curves showing the alkali reserve of different parts of sweet clover and medium red clover 361
9. Curves showing the actual and total acidities of green and of white tops of corn seedlings 363
10. Curves showing the actual and total acidities of green and of white tops of corn seedlings 364
11. Curves showing the actual and total acidities of green and of greenish-yellow tops of corn seedlings 365

THE RELATION OF SULFUR TO SOIL ACIDITY AND TO THE CONTROL OF POTATO SCAB

Fig. 1. Diagram showing the relation of sulfur treatment to hydrogen-ion concentration and the per cent of clean, salable and unsalable scabby tubers in the primes—Experiment I 398
2. Diagram showing the relation of sulfur treatments to hydrogen-ion concentration and to the per cent of clean, salable and unsalable scabby tubers in the primes—Experiment II 400
3. Diagram showing the relation of sulfur treatment to hydrogen-ion concentration and to the per cent of clean and salable scabby tubers in the primes—Experiment III 402
4. Diagram showing the relation of sulfur treatments to hydrogen-ion concentration and the per cent of clean and salable scabby tubers in the primes—Experiment I 404
5. Diagram showing the relation of sulfur treatments to hydrogen-ion concentration and the per cent of clean and salable scabby tubers in the primes—Experiment II 406

EFFECT OF VARIOUS SOLUBLE SALTS AND LIME ON EVAPORATION, CAPILLARY RISE, AND DISTRIBUTION OF WATER IN SOME AGRICULTURAL SOILS

Page

Fig. 1. Influence of various salts and lime on the evaporation of water from different soils and sand 419
2. Osmotic concentration of the soil solution of the surface inch of different soils in cylinders under various salt treatments 422
3. Moisture content of brown silt loam at different depths in cylinders as influenced by various treatments 429
4. Progress of evaporation of water from Elkton clay loam and Sassafras medium sandy loam as influenced by various treatments 432

THE PROTOZOAN FAUNA OF THE SOILS OF NEW JERSEY

C. R. FELLERS AND F. E. ALLISON

Formerly at the New Jersey Agricultural Experiment Station

Received for publication December 28, 1919

INTRODUCTION

Among the many inhabitants of the "living soil" are the protozoa. In this study other microscopic forms of life such as the algae and diatoms are included. Little do we know regarding the function in the soil of these minute organisms. What species are to be found there and how many of each? How do they get into the soil and what is their mode of life there? Does their presence influence or affect in any way the soil microflora and indirectly soil fertility in general? Some of these questions have been studied from several angles by various investigators and unfortunately often with contradictory results. The past decade, however, has contributed much valuable knowledge on this subject.

It is not the authors' intention to dwell at any length upon the literature of soil protozoa. For a critical survey of the work already done the reader is referred to the admirable review of Kopeloff and Coleman (18). Let it suffice to say that there has been to date no moderately thorough published work regarding the character of the soil microfauna. This problem constitutes the authors' principal thesis. Naturally, in the execution of such a piece of work, many interesting side-lights were encountered and studied more or less. Among these may be mentioned studies on methods and media for culture and examination, excystation, reproduction and distribution of the soil protozoan fauna. This brief report embodies the main results obtained from an extended period of research and observation of nearly two years, carried out on many soils with several media under various methods of observation and study. Most of the soils studied were examined several times at different seasons with the use of several media.

Unless one has had much experience in the routine examination and identification of plankton organisms, it requires a considerable time to become familiar enough with the literature, methods and finally the large number of species encountered, to carry on the work in a profitable manner. For this reason, much of the isolated work which has been done in the past on this subject is of limited biological value, but is important mainly from the standpoint of agricultural science and more especially soil fertility.

METHODS

In the preliminary work, soils were used from the immediate neighborhood of New Brunswick. Later samples were studied from all parts of the state. Most of the samples were taken at a depth of from 1 to 4 inches in the usual way. However, some of the samples sent in by the various county agricultural agents were put up in tin cans or paper bags. The depth of soil taken for the samples excludes the strictly surface forms. These cannot, strictly speaking, be called soil-dwellers and should be excluded in a study of this kind. Samples were taken at all seasons of the year, even in midwinter when the soil was solidly frozen. No sample was taken when there was standing water on the soil, nor were any taken in close proximity to ditches, streams, or ponds (except to study the fauna under such conditions). As many types of soil were used as possible; varying degrees of fertility and acidity were also compared to a limited extent.

One-hundred-gram portions of the soil under consideration were placed in each of three Erlenmeyer flasks containing about 125 cc. of media. The three media used generally in this work were soil extract, horse-dung and hay infusions. The flasks were incubated at room temperature (20–21°C.) and examined daily for the first 15 days, then biweekly for a period of about 2 months. In general it was found that little was to be gained from examining cultures over a month old. It is believed that all the protozoa existing in the soil as cysts or spores excyst within 30 days. A few very old cultures were studied to determine how long protozoa are able to live in the same medium. In this connection it may be stated that some forms were still found after 2 years' incubation in soil extract medium.

During the examination of cultures, care was exercised not to agitate the flasks so as to disturb the soil in them. It was found that some species were surface dwellers while others preferred the area just above the soil layer; still others were free-swimming and inhabited all parts of the liquid. The culture solution was removed from the flasks for study by means of small capillary pipettes. Both plain and hollow-ground microscopic slides were used, though the superiority of the latter is obvious. In rapid routine work, however, it is quicker to use plain glass slides. In order to find relatively rare specimens it is sometimes necessary to examine a considerable amount of material. For this work plain slides without cover slips were used, and on locating the organism, the latter was transferred to a cover slip and examined at leisure on a hollow-ground slide. For the examination of cultures a low magnification is preferable at first, then higher magnifications are used if necessary. For ordinary purposes with ciliated organisms or rhizopods the 250 and 500 magnifications are most satisfactory, but for the minute flagellates and for the study of stained specimens, it is necessary to use magnifications at times as high as 1200 diameters.

The satisfactory killing, staining and fixing of protozoa on glass slides is somewhat difficult and requires considerable patience and experience to obtain good results. Distortion of the body of the organisms is almost unavoidable. Most bacteriological stains used in connection with the dry or flame methods of fixing are not satisfactory except in the case of the minute flagellates. The killing agent must act rapidly or distortion will result. Osmic vapor or 2 per cent solution of the acid, potassium iodide, bichloride of mercury (saturated solution) to which has been added 1 per cent acetic acid, and picrosulfuric acid were the best killing reagents used. Intra-vitam staining with dilute solutions of methyl green, methylene blue or Congo red gave very good results. The Romanowsky stain as modified by Morgan (24) was tried with good results but consumed too much time for routine work. Iron haemotoxin gave good results as a nuclear stain, while for staining cilia, tannic acid was good. In the case of very active organisms it was found that observation could be much simplified by placing a few strands of cotton on the slide to restrict motility. Dilute agar solution is also useful for this purpose. For further information on the subject of staining and fixing protozoa the reader is referred to a short, concise article by Hargitt (11).

DIRECT EXAMINATION OF SOIL FOR PROTOZOA

All samples inoculated into media for study were previously studied microscopically to detect trophic protozoa. For this examination the soil sample was dampened with water, placed on a slide and examined for about 5 minutes. If no living cells were detected in that time, the sample was called negative. In but two soils collected were living protozoa found and then only in very small numbers. Samples of soil taken near ditches or ponds practically always showed living specimens, as did samples collected in low places where there was standing water after heavy rains. These latter samples are not included in the number of soils classed as normal and on which our work was based.

PRESENT STATUS OF OUR KNOWLEDGE CONCERNING THE CHARACTER OF THE SOIL FAUNA

It is interesting to speculate for a moment upon the manner in which protozoa are spread in soil. Leeuwenhoek (19) as early as 1677 found and described several species of protozoa from rain-water. The rain is therefore one of the means by which cysts were originally distributed in soils. Ehrenberg (8, 9) in 1837 mentions that he found large numbers of protozoa in *rich* soils. Since his time, Rosenburg-Lipinsky (28), Wolff (38), Cunningham and Lohnis (5), Martin (21), Waksman (34) and others mention the existence of a considerable soil fauna. No specific reference was found suggesting a plausible explanation for the presence in soil of such large numbers of species

and protozoan cells. Miquel (23) showed that protozoa and algal spores or cysts were common in air and rain-water even on the tops of the highest mountains of France. It is suggested that the air, rain, snow, winds, etc., disseminate these or ganisms over all the land. Up to the present day the validity of the old "egg and germ" theory of distribution of species by the elements mainly, has not been seriously questioned. All biologists know that protozoa are abundant on grass-stems, bark of trees, among mosses and lichens and even other places where their presence would not be expected because of the lack of moisture. Leidy (20) in his excellent treatise on rhizopods states, "Although essentially aquatic, they (rhizopods) occur wherever there is moisture, including damp rocks and soils, among vegetation, on the barks of trees, and even in the crevices of walls and pavements in cities." Pleas (25) found many living species of algae, protozoa and rotifers among lichens collected in mid-winter from dry rocks and fences. He found organisms in various stages of development and thereby concluded that the organisms had been leading a trophic existence. He calls attention to the inadequacy of the old "egg and germ" theory to account for the large numbers found. This matter will be discussed further in this paper.

The number of protozoa in soils varies widely with the nature of the soil. But there are very few soils that have not a fairly large number of cells belonging to numerous genera and species. No soil was examined in the state of New Jersey which did not contain some protozoa. The number of individuals present varies from a very few to many thousands per gram of soil. The dilution method of determining the number in soil is perhaps the most accurate method that can be used. Varying dilutions of the soil being studied, are made up and inoculated into suitable media such as soil extract or horse-dung infusion. These media are then examined microscopically for living protozoa daily up to about 30 days. It is obvious that a method which determines the number of cells in a culture solution *after* being inoculated for several days is inaccurate on account of the rapid multiplication of the organisms. Also certain species excyst after a few hours and some only after some days, hence it would be almost impossible to determine all the organisms present. By means of the dilution method the relative numbers of any species may be accurately determined although the procedure is time-consuming. Sherman (31) found some soils containing 10,000 protozoa per gram. Rahn (27) found an average of about 100 ciliates and from 1000 to 10,000 flagellates. Waksman (34) reports from 10 to 100 ciliates and 100 to 10,000 flagellates per gram of soil. In these estimates the numbers of rhizopods present in the soil are not given except very approximately. It is the authors' opinion that amoebae are very widely distributed in the soil. Since, as a rule, they excyst only after a considerable period, they are no doubt frequently overlooked. Wilson (37) substantiates this view when she refers to *naegleria* (*amoeba*) *gruberi* as the "common soil amoeba." Martin and Lewin (22) in England also found many amoebae in soils. Under ordi-

nary conditions Waksman found no protozoa at a greater depth than 12 inches and showed that the greatest numbers are concentrated in the top 4 inches of the soil.

Regarding the presence of *living* protozoa in soil, much has been written. The results reported by various investigators are conflicting. This is to be expected, however, since the character of the soil, its moisture and organic content and its bacterial and protozoan life needs must vary in different parts of the world. In general, the English investigators incline to the view that protozoa in a trophic condition are constantly present in most soils. On the other hand, the American investigators' findings have demonstrated just the reverse. Whether the English workers drew their conclusions correctly from the results obtained has been discussed in the literature. Since the very thorough work of Koch (15, 16) and Waksman (34, 35) was carried out in New Jersey, their conclusions may be applied to New Jersey soils. Koch concludes protozoa do not exist in normal soils or even in soils where the moisture content is somewhat above normal. He refers here to field soils in particular. In greenhouse soils containing much organic matter and with high moisture content he found a few living protozoa. He thinks that protozoa may become active in soils whenever the moisture content rises considerably above the normal. Waksman found very few living protozoa in field soils and this investigator shows that both moisture and organic content are the principal limiting factors for the development of protozoa in the soil. Our work is entirely in accord with that of these two investigators; namely, that in ordinary soils there are very few living protozoa even though this same soil may contain thousands of cysts per gram. In the examination of some hundred fresh samples of soil, but two were found to contain living protozoa on the direct examination. In one case several *monas guttula* were observed in a heavy clay soil of the Penn series, and in the other case a few specimens each of *naegleria gruberi* and *colpoda cucullus* were demonstrated from a shaley loam soil of the same series. In water-logged soils and in samples obtained near ditches and ponds many species of *living* protozoa were present.

Purdy and Butterfield (26), with pure, bacteria-free cultures of protozoa, have recently shown that some of the common species of protozoa cannot exist in culture solutions containing organic matter, without the presence of living bacteria. They have proven that bacteria form the main food for protozoa and that the latter cannot subsist on organic matter, as was claimed by some investigators. In culture solutions the numbers of protozoa present always increased directly at the expense of the bacteria. Several investigators using impure cultures (i.e., cultures of protozoa mixed with bacteria), have shown that protozoa are bacteria eaters. Among these are Huntemüller (13) and Hoffman (12), who showed conclusively that certain water protozoa ingest typhoid bacteria. Among the species mentioned are two common soil flagelates, viz., *bodo ovatus* and *bodo saltans*. This is of interest, as Sherman (31)

in his extended researches found that flagellates did not limit bacterial numbers in solution and soil. He found that the ciliates were capable of causing a large decrease in numbers of bacteria in culture solutions and in soil under proper conditions. Waksman's (35) results are in general in accord with Sherman's. It is thus seen that at least some species of protozoa use bacteria regularly as food, and it follows that the protozoa *living* in the soil also ingest bacteria. Since flagellates are the most common of all the protozoa and as Sherman found in general that they did not consume many bacteria, they are not perhaps of much consequence in the soil. However, this point needs to be more thoroughly investigated before any conclusions can be safely drawn. Goodey (10) shows that active protozoa exist in certain sewage-treated soils in England and may be a limiting factor in the fertility of those soils. This is undoubtedly true, and even in some of the wet, low-lying soils of New Jersey, the protozoa present may have a considerable detrimental influence upon the bacteria which are present. Russel and Huchinson (29, 30) claim that when soils are partially sterilized by heat or antiseptics the beneficial effect noted on the subsequent crop is a result of the destruction of an injurious biological factor (protozoa supposedly). Much controversy has arisen concerning this veiwpoint but it is probably the consensus of opinion of soil biologists that the well-known beneficial effect upon the soil is due to a number of other reasons. Under certain conditions it cannot be denied that protozoa might be able to decrease the fertility of a soil by destroying the beneficial soil bacteria.

ENUMERATION OF SPECIES OF PROTOZOA AND ALGAE IN NEW JERSEY SOILS

For the identification of these organisms, great care was taken to consult thoroughly the literature of protozoölogy to make certain of the correct nomenclature. In some cases this has been found to be very difficult because of the often imperfect descriptions and figures of the early investigators combined with the over-zealousness of others in making new genera and species when existing classifications were entirely adequate. It is not uncommon for a single organism to be described in the literature under five or six different names. In compiling the list below, the name of the investigator who named the species is always mentioned so that no doubt may be entertained as to the organism described. As the literature on the subject is somewhat scattered, some of the more useful works found for the identification of these organisms are given in the bibliography. Among them are Butschli (1), Kent (14), Calkins (2, 3), Döflein (6), Leidy (20), Stokes (33), Conn (4), Edmondson (7), Stein (32), Whipple (36) and Wolle (39, 40).

In the plates appended to this paper the more common soil protozoa and algae are roughly pictured. The drawings were originally made from the living and stained organisms themselves. In a few of the drawings, figures taken from the literature were used in connection with the original drawings.

The following species were identified.[1]

A list of the protozoa and algae found in New Jersey soils

(For drawings of the commoner forms see plates 1–4)

Class—Sarcodina
Subclass—Rhizopoda
- Hyalodiscus (amoeba) limax Duj. ++
- Hyalodiscus (amoeba) guttula Duj. ++
- Amoeba proteus Ehrbg. ++
- Amoeba radiosa Ehrbg. ++
- Naegleria (amoeba) gruberi Schardinger (Wilson) ++++
- Arcella vulgaris Ehrbg. +
- Difflugia pyriformis Perty +
- Difflugia globulosa Duj. +
- Pamphagus mutabilis Bailey +
- Microgromia socialis Arch. +
- Diplophrys archeri Bark. +
- [2]Mastigamoeba sp. (?) ++
- [2]Ciliophrys infusionum Cienk. +

Subclass—Heliozoa
- Actinophrys sol Ehrbg. ++
- Vampyrella lateritia Fres. +
- Raphidiophrys sp. (?) +
- Clathrulina elegans Cienk. +

Class—Mastigophora
Subclass—Flagellidia
- Monas termo Ehrbg. (Martin) ++++
- Monas guttula Ehrbg. +++
- Monas vivipara Ehrbg. ++
- Oikomonas mutabilis S. K. (Kent) +++
- Cercemonas crassicauda Duj. ++++
- Cercemonas longicauda Duj. +++
- Pleuromonas jaculans Perty ++
- Physomonas elongata Stokes ++
- Monosiga ovata S. K. +
- Phyllomitis amylophagus (?) Klebs ++
- Bodo (heteromita) ovatus (caudatus) S. K. +++
- Bodo globosus Stein ++
- Bodo lens Müll. (S. K.) +
- Bodo sp. (?). Resembles Conn No. 79 +
- Clostenema socialis (?) Stokes +
- Tetramitis variabilis Stokes +
- Hexamitis inflatus Duj. +
- Euglena viridis Ehrbg. ++
- Euglena acus Ehrbg. ++
- Phacus pyrum Ehrbg. ++

---

[1] Relative abundance of species is indicated by plus (+) marks as follows: rare +; occasional ++; common +++; very common ++++. Identification doubtful (?).

[2] These two forms are often included in the Mastigophora.

Class—Mastigophora
Subclass—Flagellidia—*Continued*
Phacus longicaudus Ehrbg. +
Eutrepia viridis (?) Perty +
Trachelomonas volvocina Ehrbg. +
Cryptoglena pigra Ehrbg. ++
Astasia trichophora (?) Ehrbg. ++
Astasia sp. (?). Resembles Conn No. 73 +
Paranema trichophora Ehrbg. ++
Heteronema acus Ehrbg. ++
Petalomonas mediocanellata (?) Stein +
Mallomonas sp. (?). Resembles Edm. No. 85 +
Crytomonas ovata Ehrbg. ++
Chilomonas paramecium Ehrbg. (Stokes) ++
Chilomonas sp. (?). Resembles Conn No. 95 +
Chlamydomonas sp. (?) ++

Class—Infusoria
Subclass—Ciliata
Holophrya sp. (?). Resembles Conn No. 120 +++
Holophyra sp. (?). Resembles Conn No. 121 ++
Urotricha sp. (?). Resembles Edm. No. 91 ++
Urotricha (balantozoon) agile Stokes ++
Enchelys farcimen Ehrbg. ++++
Enchelys sp. (?). Resembles Conn No. 124 ++
Prorodon teres (?) Ehrbg. ++
Prorodon armatus (?) C. & L. ++
Mesodinium sp. (?) +
Lionotus fasciola Ehrbg. ++
Loxophyllum flexilis Stokes ++
Dileptus gigas C. & L. ++
Dileptus sp. (?). Resembles Conn No. 160 ++
Chilidon cucullulus Müll. ++
Chilidon megalotrochae Stokes +++
Trichoda pura Ehrbg. (Edm. No. 118) ++
Glaucoma scintillans Ehrbg. +++
Colpidium striatum Stokes +++
Colpidium colpoda (?) Stein +++
Uronema marina Duj. ++
Colpoda (tillina) saprophila Stokes +++
Colpoda cucullus Stein ++++
Colpoda campyla Stokes +++
Colpoda helia Stokes ++
Colpoda flavicans (?) Stokes +
Paramoecium trichium Stokes +
Paramoecium caudatum Ehrbg. +
Pleuronema chrysalis Ehrbg. ++
Pleuronema sp. (?). Resembles Conn No. 211 ++
Cyclidium glaucoma Ehrbg. +++
Lembus pusillus (?) Quenn. (Calk.) +
Blepharisma (apgaria) ovata (?) Stokes +
Metopus sigmoides C. & L. ++
Metopides acuminata (?) Stokes +

Class—Infusoria
Subclass—Ciliata—*Continued*

Strombidium sp. (?). Resembles Conn No. 229 +
Halteria grandinella O. F. Müll. +++
Stichotrica secunda Perty +
Uroleptus dispar (?) Stokes ++
Uroleptus musculus (?) Ehrbg. +
Oxytricha pellionella Müll. +++
Oxytricha bifaria Stokes ++
Stylonichia mytilis Ehrbg. +++
Stylonichia pustulata Ehrbg. ++
Euplotes charon Müll. (Ehrbg.) ++
Euplotes carinata Stokes +
Aspidisca costata Duj. ++
Vorticella striata Duj. ++++
Vorticella citrina Ehrbg. +++
Vorticella putrina Müll. ++
Vorticella microstoma Ehrbg. +++
Vorticella globularia Müll. ++

Soil algae and diatoms

Protococcus viridis Ag. +++
Protococcus sp. (?) +++
Botryococcus sp. (?) +
Tetraspora sp. (?) ++
Chroöcoccus cohoerens (?) Naeg. ++
Nostoc sp. (?) ++
Closterium sp. (?) ++
Cosmarium sp. (?) +
Coelosphaerium sp. (?) +
Ulothrix sp. (?) ++
Spirogyra sp. (?) +

Diatoma vulgare ++
Navicula rhyncocephara (?) ++
Stauroneis sp. (?) +
Cymbella sp. (?) ++
Nitzchia sp. (?) ++
Synedra sp. (?). +

Discussion

Contained in this list are 13 species of rhizopods, 4 heliozoa, 34 flagellates, 51 ciliates; and in addition to the protozoa are 11 algae and 6 diatoms. It is thus seen that from a species standpoint, the cilates are most numerous, followed in order by the flagellates, rhizopods and heliozoa. In point of actual numbers in the soil, however, flagellates easily rank first, followed by the ciliates and rhizopods. The larger size of the ciliates tends to diminish the advantage which the flagellates have from the standpoint of numbers. Comparing these results with those obtained by Kofoid (17) in his study of the plankton of the Illinois, Spoon and Quiver rivers we find fair agreement.

In the Illinois river he found 30 rhizopods, 4 heliozoa, 62 mastigophora, 55 ciliata and 5 suctoria. In the Spoon River he found 17 rhizopods, no heliozoa, 38 mastigophora, 16 ciliata and 2 suctoria, while in the Quiver River the only organisms found were 13 rhizopods and 5 mastigophora. With respect to species relationship, then, the soil microfauna is not unlike that of fresh water. The main points of difference are in the absence from, or very limited presence in soil of such forms as the suctoria, rotifera and crustacea. It appears that they cannot withstand the desiccation and other unfavorable conditions which exist in soil.

Compiling those species which have been classed as abundant or common in New Jersey soils we find that there are 17 ciliates, 6 flagellates, 1 rhizopod and 1 alga. Seven of the cilates, however, belong to two genera, namely *colpoda* and *vorticella*. As regards abundant species the ciliates are again first, with flagellates second. Only 6 species are classed as "very abundant." These are *naegleria gruberi, monas termo, cercemonas crassicauda, enchelys farcimen, colpoda cucullus* and *vorticella striata*. Several other genera, such as the *bodos, monas, halteria, colpidium* and *stylonichia* were almost as common.

Of course, a considerable number of organisms were encountered which could not be even tentatively identified. Several fairly common ciliates and flagellates fall into this class. Some are undoubtedly new species and some may even have to be given new generic names. The soil protozoa would furnish the systematist with rich material for investigational work.

The algae and diatoms were not thoroughly studied but are mentioned in this connection mainly because of their close relationship. The algae belong mainly to the coccus forms although a few filamentous genera were found. In order to cultivate the algae in soil, the latter was mixed with quartz sand in a flask, saturated with a weak soil extract and left in the sunlight for several months. Under these conditions the algae present will form a mat on the surface of the soil-sand mixture. Soil algae may be of importance in soil fertility by furnishing energy material for the growth of certain beneficial soil bacteria such as the *azotobacter*. By virtue of the ability of algae to manufacture chlorophyll in the presence of sunlight and of the ability of *azotobacter* to fix atmospheric nitrogen, a mutual beneficial association of these two organisms might result. Beijerinck, in Holland, has shown that such an association actually does take place in the soil; the algae furnishing the necessary carbohydrate and the bacteria, the nitrogenous matter. D. A. Coleman, in unpublished data, has found that *nostoc* incubated in flasks in the sunlight with several mixtures of *azotobacter* does not furnish appreciable energy-material for the fixation of atmospheric nitrogen. Of course, in the field conditions may be such that appreciable fixation takes place.

To throw some light upon the question of the similarity of the microfauna of the soil and that of neighboring ditches, pools and ponds, many samples taken from the latter sources were studied. In general, the fauna was much

more varied in character than that of the soil. Rotifers, crustaceans, diatoms, suctoria, sponges and certain colonial protozoa were to be found in the fresh-water ponds and streams and not in the soil. In both soil and water, nematodes and larvae of insects were often encountered. However, nearly all species which were found in soil in New Jersey have been also identified from fresh-water sources either in New York or in New Jersey. Their relative abundance, however, is not necessarily the same in soil and water; in fact it is to be noted that some very common plankton organisms were never found in the soil, and *vice versa*, some of the common soil protozoa are not often found in fresh-water. The work of Stokes (33) in New Jersey and other states, Conn (4) in Connecticut, Edmondson (7) in Iowa, and Kofoid (17) in Illinois, on the fresh-water species of protozoa, shows that the same organisms are present in nearly every locality but not in the same relative abundance. This apparently is true also in the case of soils.

It was plainly noticeable that many of the terrestrial protozoa and algae often differed materially in size, color and in some other characteristics from the same organism taken from fresh or slightly brackish water. Sometimes a sheath or membrane was absent, a green form would be colorless and so-called "social species" would be "solitary" in the soil. That environment has brought about these changes seems evident. This observation also materially strengthens the conjecture that there is a fairly definite soil microfauna and that the protozoa lead a trophic existence whenever soil moisture becomes excessive. Such a condition takes place after heavy rains, floods and in the spring when frozen soil is thawing out.

The characteristic protozoa of the soil, then, are those simple, small, hardy forms which are capable by means of rapid encystation or otherwise, to withstand successfully extremes of heat and cold, desiccation, aeration and such other inhibiting factors as are natural to their home in the soil.

DISTRIBUTION OF PROTOZOA IN DIFFERENT SOILS

Thirty-five soils were collected for a study of the distribution of protozoa in different soils. All but three of these were typical New Jersey soils and represented nearly every county in the state. The work of identification of species was naturally incomplete, for the reason that sufficient time could not be given to any one soil sample to insure getting every organism there. Two media were used—soil extract and horse-dung. One-hundred-gram portions of each soil were used in each of the two media. In this connection it should be stated that of five media tested out for soil protozoa, namely, blood-meal extract, 1 per cent beef broth, hay infusion, soil extract (heated in an autoclave), and horse-dung infusion, the latter two were found to be best suited to our work. The main difficulty with the others was that bacterial growth was so heavy as to prevent the development of the protozoa; the solutions often becoming putrescent. The flasks were examined daily for two weeks,

TABLE 1

Group relationship and distribution of protozoa in soils

SOILS	FLAGELLIDIA	CILIATA	RHIZOPODA	HELIOZOA	DIATOMS	ALGAE	TOTAL SPECIES OF PROTOZOA*
1A. Middlesex County	8	8	1	0	1	1	17
3A. Middlesex County	8	16	3	1	0	0	28
5A. Middlesex County	4	10	6	0	0	1	20
6A. Middlesex County	6	10	2	0	0	0	18
7A. Middlesex County	3	5	1	0	2	1	9
8A. Middlesex County	11	13	0	1	0	3	25
Sussex County	3	7	2	0	0	0	12
Burlington County	6	2	2	0	0	0	10
Monmouth County	9	7	2	1	0	0	19
Cumberland County	7	6	0	0	0	1	13
Atlantic County	6	11	3	3	1	2	23
Cape May County	1	1	0	0	0	0	2
Morris County	3	2	0	0	0	0	6
Hunterdon County	2	1	1	0	0	0	4
Warren County	6	5	0	0	0	0	11
Union County	1	2	1	0	0	0	4
Ocean County	1	4	0	0	0	0	5
Bergen County	6	5	0	0	0	0	11
Somerset County	6	5	1	0	0	0	12
Mercer County	7	1	1	0	1	0	9
Essex County	2	3	1	0	0	1	6
Camden County	4	2	0	0	1	0	6
Passaic County	10	5	3	1	0	2	19
1. Middlesex County	2	5	1	0	0	0	8
2. Middlesex County	1	0	1	0	0	0	2
3. Middlesex County	2	2	0	1	0	0	5
4. Middlesex County	2	5	1	0	1	1	8
5. Middlesex County	4	13	1	1	0	2	19
6. Middlesex County	8	5	1	0	0	0	14
7. Middlesex County	4	7	0	0	0	0	11
8. Middlesex County	8	8	2	0	1	0	18
9. Middlesex County	2	4	0	0	0	0	6
10. Middlesex County	4	3	1	1	3	0	9
Colorado (normal)	3	3	0	0	0	0	6
Colorado (nitre spot)	1	3	0	0	0	0	4
Florida (sandy)	2	1	2	0	0	0	5
Totals (New Jersey soils only)*	157	184	38	10	11	15	389
Average number of species per soil (nearest integer)	5	6	1	Less than 1	Less than 1	Less than 1	12

* Algae and diatoms not included in totals.

then semi-weekly for two weeks more. This series of samples was examined for evidence of living protozoa but with negative results. In some cases, however, the samples had partly dried out by the time they arrived at the laboratory, and had there been any free living organisms in the fresh soil they might have encysted again.

Discussion

The results given in table 1 indicate that protozoa are present in some form or other in all the soils examined. The largest number of species identified from any one soil was 28, this large number being obtained from a fertile but heavy clay of the Penn series. The smallest number of species found in any soil was 2, in both an acid virgin forest soil from Middlesex County and a coarse sandy soil from Cape May County. In general it seemed to make but little difference where the samples of soil were obtained —a fair number of organisms were always secured. Certain species, however, appeared only in certain soils, for example *dileptus gigas* and *uroleptus musculus* were found only in Sussex County soils, *euglena acus*, in a rich black forest soil of Middlesex County, and *difflugia pyriformis* as well as *vampyrella lateritia* were identified from but one locality in Middlesex County. Similar observations have been made regarding the distribution of certain fresh-water organisms. In certain pools one may always find certain species, yet again he can search for days in other localities and not encounter them. This unique circumstance regarding distribution of species in fresh water apparently holds also in the case of the soil microfauna.

RELATION OF PROTOZOA TO SOIL TYPE, SOIL BACTERIA AND MOLDS

This relation was studied only in a very incomplete manner, but as some facts of interest were indicated by the work, it is here reported. It was generally observed that the soils apparently in a high state of fertility practically always showed the presence of more species and numbers of organisms than did the less fertile soils. To test out this observation further the following data are offered. More direct evidence is required, however, before the question can be definitely decided.

The data contained in table 2 show that of the soils studied, those in a high state of fertility harbored the greatest number of protozoa as well as the greatest number of species. The same is also true regarding the number of bacteria present. Molds do not apparently follow this general rule for nearly as many were noted in an acid soil under sod as in a virgin forest soil. The acid soil had received large amounts of ammonium sulfate annually for some years previous. The forest soil was also acid. The other soils tested were either only very slightly acid or neutral in reaction. Field conditions seem to show that the same relation holds between numbers of protozoa and bacteria in the soil as well as in the culture flask. In other words, where

there are the greatest numbers of bacteria, there also do we find the greatest number of protozoan cells and species. . Such a statement must be broadly interpreted, as it is reasonable to suppose that there are many instances where this relation does not hold. For example, very wet soils may be very low in numbers of bacteria but still contain many protozoa, also where bacterial putrefaction is taking place and large numbers of bacteria are present, protozoa may be almost entirely inhibited. Such a condition is often observed in culture flasks containing blood meal or beef extract. In many cases, however, a high bacteria count and high protozoa count (or at least a large number of species) go together. This might be explained by supposing that during the periods of their activity in the soil the protozoa find great quantities of food and are thus able to propagate rapidly while conditions are favorable. As the soil dries out they go into encystment again. This seems to be the logical explanation of the facts, especially since Goodey (10) and others have shown that encystation and excystation can take place in a few hours under suitable conditions.

TABLE 2

SOURCE OF SOIL SAMPLES	NUMBER OF PROTOZOA PER GRAM*	NUMBER OF BACTERIA PER GRAM	NUMBER OF MOLDS PER GRAM	NUMBER OF SPECIES OF PROTOZOA FOUND IN SAMPLE
Cultivated rich garden soil.....	3500	5,300,000	125,000	26
Alfalfa sod (rich).....	4500	5,000,000	90,000	23
Cultivated orchard (poor).....	1000	4,850,000	85,000	9
Very acid soil (sod).....	Very few	960,000	138,000	4
Virgin forest.....	55	1,500,000	110,000	3

* The data in this column were obtained from Dr. S. A. Waksman.

IS THERE A TRUE SOIL MICROFAUNA?

If we consider this question in the light that the soil protozoa are distinct and different species altogether from the fresh-water forms, then we must say that there is none. But, on the other hand, if we mean by "true soil microfauna," those organisms which are practically always present in the soil in considerable numbers and which use the soil as a medium in which to live and carry on their life processes, we must say that there is. It has been observed that just because an organism may be extremely abundant in fresh water it does not necessarily follow that it is also common in soil. The converse of this statement also is true. A striking example of this nature is in the case of *paramoecium caudatum*. This large easily identified protozoan is extremely abundant in fresh water, yet it is but rarely encountered in New Jersey soils. *Naegleria gruberi*, the commonest soil rhizopod found, is encountered only occasionally in plankton studies. In general, we may say that a considerable number of species of protozoa are present in fairly large numbers in most soils. Certain species of algae also seem to be common in

soil; the most common form being the *protococcus*. As *rotifera* and the more complex plankton organisms, with the exception of nematodes, do not occur except very rarely in soils, they have little significance in this consideration.

SUMMARY

1. Protozoa were found in all the soils examined; the number of species identified from any one soil varying from 2 to 28. Poor sandy acid or forest soils contained the least number of species, while fertile soils, rich in organic matter or with high water-holding capacity, contained the greatest number.

2. Of the 104 species identified from New Jersey soils, 51 were ciliates, 35 were flagellates, 14 were rhizopods and 4 were heliozoa. In addition to the protozoa 10 genera of algae and 6 of diatoms were identified. Nematodes were common, but rotifers or crustaceans were not encountered.

3. Of the species classed as being abundant in the soil, 17 were ciliates, 6 were flagellates and 1 a rhizopod. The ciliates outnumber the flagellates in the number of species identified but *not* in actual numbers of organisms in the soil. In point of numbers the rhizopods and heliozoa rank third and fourth, respectively. No suctoria were encountered.

4. It is believed that in normal[3] New Jersey soils protozoa exist mainly in a non-trophic state. Only two soils out of some hundred samples taken all over the state showed the presence of living protozoa on direct examination. In soils which are saturated with water for several hours, or in standing soil-water, field ditches and plough-furrows, limited numbers of protozoa may usually be found. Since their activity is very limited, especially at low temperatures, they can have but little importance from the standpoint of soil fertility under New Jersey conditions.

5. The soil microfauna consists principally of those small, simple and hardy protozoa which are able to withstand by means of encystation or otherwise, such extremes of heat and cold, desiccation, aeration, etc., as are natural to their life in the soil.

6. Practically all the species which were identified from the soil have also been found in the fresh-water lakes, ponds, pools and streams of the state, but *not* in the same relative abundance. Some species which are abundant in soil are rarely encountered in fresh water, and *vice versa*, several of the most common plankton organisms in the state are but rarely found in soil.

7. A new theory to replace the old "egg and germ" theory of distribution of protozoa in soil is put forth as follows:

Some protozoa undoubtedly are spread about by rain, winds, animals and other natural forces. It is not believed that these means are sufficient to account for the large number of organisms of many species found in all soils. Protozoa become active in soil whenever there is excessive moisture present for a period of several hours. Such conditions are common during prolonged

[3] Soils not containing moisture much in excess of the physical optimum.

rains especially in the rainy seasons, during winter and spring thaws, in soils kept moist from seepage and in poorly drained lands. Excystation takes place followed by a feeding period and consequent growth, then reproduction takes place. As conditions become unfavorable again, encystation begins or death occurs. Since all these processes may take place in the course of a few hours (but usually requiring 24 hours at least for appreciable reproduction to take place), the whole life cycle of many protozoa may be completed in the soil.

In support of this contention it may be stated that there is apparently a fairly definite soil fauna which differs in several respects from the surrounding fresh-water fauna. Rich, fertile soils contain greater numbers of both bacteria and protozoa than poor or very acid soils. Since protozoa use the bacteria as food it seems reasonable that they would multiply more readily in places where there is plenty of food. Protozoa may be generally found in field ditches, plow furrows, in soil where there is standing water, and in soils wet from seepage, showing that they may lead a trophic existence in soil if conditions are suitable. It is absolutely unreasonable to conceive of the elements and other natural forces as being accountable for 5000 protozoa in practically every gram of soil in the state.

REFERENCES

(1) Bütschli, O. 1880–1889 Protozoa Klassen und Ordnungen des Thier-Reichs. Abt. I–II–III. (Bronn, H. C.)

(2) Calkins, G. N. 1901 The Protozoa. 347 p. Macmillan Co., New York.

(3) Calkins, G. N. 1901 Marine protozoa from Woods Hole. *In* U. S. Fish Com. Bul. (1901), p. 413–468.

(4) Conn, H. W. 1905 A preliminary report of the protozoa of the fresh waters of Connecticut. Conn. State Geol. Nat. Hist. Survey, Bul. 2.

(5) Cunningham, A., and Lohnis, F. 1914 Studies on soil protozoa. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 39, No. 23–25, p. 596–610.

(6) Döflein, E. 1909 Lehrbuch der Protozoenkunde. Gustav Fisher, Jena.

(7) Edmondson, C. H. 1906 The protozoa of Iowa. *In* Proc. Davenport Acad. Sci., v. 11, p. 1–124.

(8) Ehrenberg, C. G. 1837 Die fossilen Infusorien und lebendige Dammerde. 27 p. Berlin.

(9) Ehrenburg, C. G. 1838 Die Infusionsthierchen als volkommene Organismen. 532 p.

(10) Goodey, T. 1915 Investigations on protozoa in relation to the factor limiting bacteria activity in soil *In* Proc. Roy. Soc. (London), s. B., v. 88, no. B606, p. 437–456.

(11) Hargitt, C. W. 1899 Methods of studying and mounting protozoa. *In* Jour. Appl. Micros., v. 2 no. 6, p. 385–388.

(12) Hoffman, W. 1905 Untersuchungen über die Lebensdauer von Typhusbazillen im Aquariumwasser. *In* Arch. Hyg., Bd. 52, p. 208–217.

(13) Huntermüller, O. 1905 Vernichtung von Bakterien im Wasser durch Protozoen. *In* Arch. Hyg., Bd. 54, p. 89–100.

(14) Kent, W. S. 1880–1882 A Manual of Infusoria. 2 vol. David Bogue, London.

(15) KOCH, G. P. 1915 Activity of soil protozoa. *In* Jour. Agr. Res., v. 5, no. 11, p. 477–488.

(16) KOCH, G. P. 1916 Studies on the activity of soil protozoa. *In* Soil Sci., v. 2, no. 2, p. 163.

(17) KOFOID, C. A. 1904 (1908) The plankton of the Illinois River: Part I. *In* Bul. Ill. State Lab. Nat. Hist., v. 6, p. 95–629; Part II. *In* Bul. Ill. State Lab. Nat. Hist., v. 8, p. 1–361.

(18) KOPELOFF, N., and COLEMAN, D. A. 1917 A review of investigations in soil protozoa and soil sterilization. *In* Soil Sci., v. 3, no. 3, p. 197–269.

(19) LEEUWENHOEK, A. VAN 1677 Observations concerning little animals observed in rain, well, sea and snow water and also in water wherein pepper had lain infused. *In* Phil. Trans. Roy. Soc. London, v. 12, no. 133.

(20) LEIDY, J. 1879 Fresh-water rhizopods of North America. *In* Rpt. U. S. Geol. Survey, v. 12, 324 p.

(21) MARTIN, C. H. 1912 A note on protozoa from sick soils, (etc.). *In* Proc. Roy. Soc. (London), s. B, v. 85, no. B580, p. 393–400.

(22) MARTIN, C. H., AND LEWIN, K. R. 1914 Some notes on soil protozoa. *In* Phil. Trans. Roy. Soc. London, s. B, v. 205, p. 77–94.

(23) MIQUEL, P. 1879 Etude sur les pousseières organisées. *In* Ann. Observ. Mont-Souris (1879), p. 431.

(24) MORGAN, J. F. 1915 The staining of soil protozoa. *In* Mich. State Bd. Agr. 17th Ann. Rpt., p. 25–27.

(25) PLEAS, E. 1896 Occurrence of several families of aquatic animaliculae in new stations. *In* Trans. Wis. Acad. Sci., Arts, and Letters, v. 10, p. 421.

(26) PURDY, W. C., AND BUTTERFIELD, C. T. 1918 The effect of plankton animals upon bacterial death-rates. *In* Amer. Jour. Pub. Health, v. 8, no. 7, p. 499–505.

(27) RAHN, O. 1913 Methode zur Schatzung der Anzahl von Protozoen im Boden. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 36, p. 419–421.

(28) ROSENBERG-LIPINSKY, ALB. v. 1869 Der Praktische Akerbau (etc.), Bd. 2, p. 27. Breslau.

(29) RUSSEL, E. J., AND HUCHINSON, H. B. 1909 The effect of partial sterilization of soil on the production of plant-food. *In* Jour. Agr. Sci., v. 3, pt. 2, p. 111–144.

(30) RUSSEL, E. J., AND HUCHINSON, H. B. 1913 The effect of partial sterilization of soil on the production of plant food. II. The limitation of bacterial numbers in soil and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152–221.

(31) SHERMAN, J. M. 1916 Studies on soil protozoa and their relation to the bacterial flora. Part I. *In* Jour. Bact., v. 1, no. 1, p. 35–66. Part II. *In* Jour. Bact., v. 1, no. 2, p. 165–185.

(32) STEIN, F. 1859–1878 Der Organismus der Infusionsthiere. 3 vol. Leipzig.

(33) STOKES, A. C. 1888 A preliminary contribution towards a history of the fresh-water infusoria of the United States. *In* Jour. Trenton Nat. Hist. Soc., v. 1, p. 79–319.

(34) WAKSMAN, S. A. 1916 Studies on soil protozoa. *In* Soil Sci., v. 1, no. 2, p. 135–152.

(35) WAKSMAN, S. A. 1916 Protozoa as affecting bacterial activity in soils. *In* Soil Sci., v. 2, no. 4, p. 463.

(36) WHIPPLE, G. C. 1914 The microscopy of drinking water. 409 p. John Wiley and Sons, New York.

(37) WILSON, C. W. 1916 On the life history of a soil amoeba. *In* Univ. Cal. Pub. Zool., v. 16, no. 16, p. 292–341.

(38) WOLFF, M. 1912 Über Bodenprotozoen. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 33, p. 314–320.

(39) WOLLE, F. 1887 Fresh water algae of the United States, 2 vol. 364 p. Comenius Press, Bethlehem, Pa.

(40) WOLLE, F. 1890 Diatomaceae of North America. Comenius Press, Bethlehem, Pa.

PLATE 1

Rhizopoda

1. *Hyalodiocus limax* Duj. 30–80 μ (45), occasional; simple, hyaline with single pseudopodium.

2a. *Hyalodiscus* (*amoeba*) *guttula* Duj. 10–25 μ (20); occasional; a very minute rhizopod; usually club-shaped; endoplasm clear with few large granules.

2b. Same, with pseudopodium extended.

3. *Amoeba proteus* Ehrbg. 70–250 μ (150); occasional; a large many-lobed organism.

4a. *Naegleria* (*amoeba*) *gruberi*, Schardinger (Wilson). 15–40 μ (25); trophozoite; very abundant; drawing shows nuclear change taking place.

4b. Same; one pseudopod extended while the other is retracted.

4c. Same; flagellated stage 15–30 μ (20); this stage found only occasionally in cultures but often on exposed microscopic slides.

5a. *Amoeba radiosa* Ehrbg. Body 15–35 μ (18); occasional; this is usual form.

5b. Same; radial spikes having changed into pseudopodia.

6. *Arcella vulgaris* Ehrbg. 50–90 μ (70); occasional; top-view showing delicate finger-like pseudopodia beneath the shell.

7. *Mastigamoeba* F. E. Sch. sp.? 15–35 μ (30); occasional; short, blunt, unbranched pseudopodia. Usually classed with the *Mastigophora* on account of the flagellum. A variable genus.

8. *Difflugia pyriformis* Perty. 40–200 μ (90); occasional; slender, delicate pseudopodia extending from beneath the silicious shell.

9. *Microgromia socialis* Arch. Rare. Individuals vary in size from 8–14 μ (12); a colonial form; pseudopodia not anastomosing in some specimens.

10. *Actinophrys sol* Ehrbg. 50–100 μ (70); occasional; specimen much vacuolated; one of the commonest *Heliozoa* found in the soil.

11. *Diplophrys archeri* Bark. 20–35 μ (25); rare, showing several contractile vacuoles and oil globules.

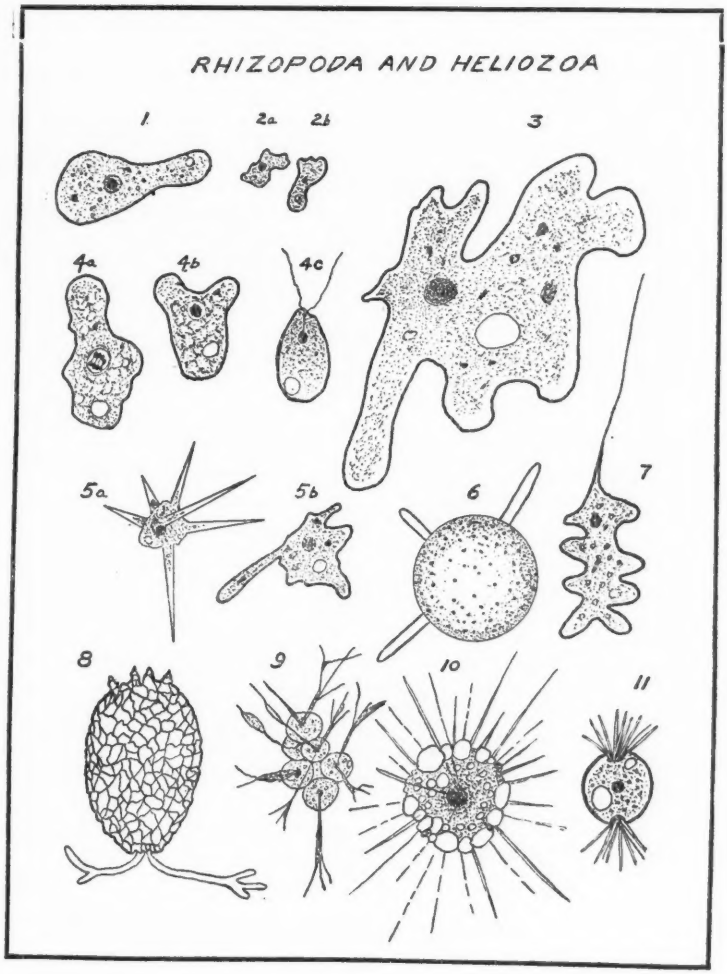

RHIZOPODA AND HELIOZOA
1
2a
2b
3
4a
4b
4c
5a
5b
6
7
8
9
10
11

PLATE 2

Mastigophora (Flagellates)

1. *Monas termo* Ehrbg. (Martin); 5–10 μ (7); very abundant; usual shape globular to ovoid.

1a. Same; ovoid form of this species; body somewhat changeable in shape.

1b. Same; showing this organism with 2 flagella ready to divide.

2. *Monas guttula* Ehrbg. 7–15 μ (12); abundant; posterior nucleus and posterior vacuole.

2a. Same; semi-amoeboid form showing 2 dissimilar flagella.

3. *Monas vivipara* Ehrbg. (Stein); 12–25 μ (17); sometimes attached at base; occasional.

3a. Same; showing a common amoeboid form; nucleus visible with reagents.

4. *Oikomonas mutabilis* S. K. (Kent); 7–15 μ (10); abundant; sometimes attached at base.

4a. Same; showing a specimen with distorted body and single flagellum.

5. *Cercemonas crassicauda* Duj. 10–25 μ (18); very abundant; shape variable.

5a. Same; semi-amoeboid form; flagella sometimes appear as simply body prolongations.

6. *Physomonas elongata* Stokes. 7–15 μ (9); occasional; body usually twice as long as broad.

6a. Same; showing changeable shape; sometimes attached by a posterior pedicel.

7. *Pleuromonas jaculans* Perty. 5–11 μ (8); occasional; kidney-shaped to spherical.

7a. Same; showing a more spherical form attached by posterior flagella; nucleus, anterior.

8. *Mallomonas*, Perty, sp. Edm.? 12–28 μ (18); rare; body covered with rigid hairs or setae.

9. *Phyllomitis amylophagus* Klebs (?), Conn. 10–22 μ (16); occasional; one flagellum often trailing.

9a. Same; a smaller form with a more flexible body.

10. *Bodo ovatus* Duj. Average size 14 μ x 8 μ; abundant; one flagellum trailing while the other is used as a propulsive whip anteriorly.

11. *Bodo globosus* Stein. 8–12 μ (9); abundant; almost spherical in form.

12. *Bodo*, Ehrbg. sp. (?); *Heteromita* (Stokes). 10–14 μ (11); occasional; an oblique, longitudinally ridged specimen.

13. *Cryptomonas ovata* Ehrbg. 20–250 μ (30); occasional; specimen shows green chlorophyll bands encircling the body.

14. *Chlamydomonas*, Ehrbg. sp. (?). 12–30 μ (20); occasional; endoplasm green; single eyespot.

15. *Trachelomonas volvocina* Ehrbg. 25–50 μ (35); occasional; color usually brown; sometimes without neck-like projection.

16. *Cryptoglena pigra* Ehrbg. 10–20 μ (13); occasional; 2 pigment bands and single eyespot.

17. *Chilomonas paramecium* Ehrbg. (Stokes); 20–40 μ (28); occasional; vacuole located anteriorally; dark colored corpuscles scattered in the endoplasm.

18. *Monosiga ovata* S. K. 10–15 μ (12); rare; solitary; often attached to debris.

19. *Hexamitus inflatus* Duj. 12–22 μ (15); rare; 4 vibratile anterior and 2 posterior trailing flagella.

20. *Phacus pyrum* Ehrbg. 15–50 μ (25); occasional; color greenish; eyespot; body obliquely grooved.

21. *Euglena viridis* Ehrbg. 18–60 μ (30); occasional; body usually green; red eyespot; body shape variable; organism senstive to stimuli.

22. *Paranema trichophora* Ehrbg. 50–120 μ (90); occasional; body very flexible; nucleus central.

23. *Heteronema acus* Ehrbg. (Stein). 35–80 μ (50); occasional; body plastic; longer flagellum trailing.

24. *Astasia trichophora* Ehrbg. (?) 30–60 μ (45); occasional; body plastic; endoplasm is transparent; no eyespot present.

24a. Same; showing a commonly observed distortion.

25. *Tetramitus variabilis* Stokes. 10–20 μ (16); rare; 4 subequal flagella; body obovate plastic and variable in shape.

26. *Euglena acus Ehrbg.* 40–100 μ (70); occasional; body very elongate; greenish in color; posterior end pointed; nucleus central; eyespot present.

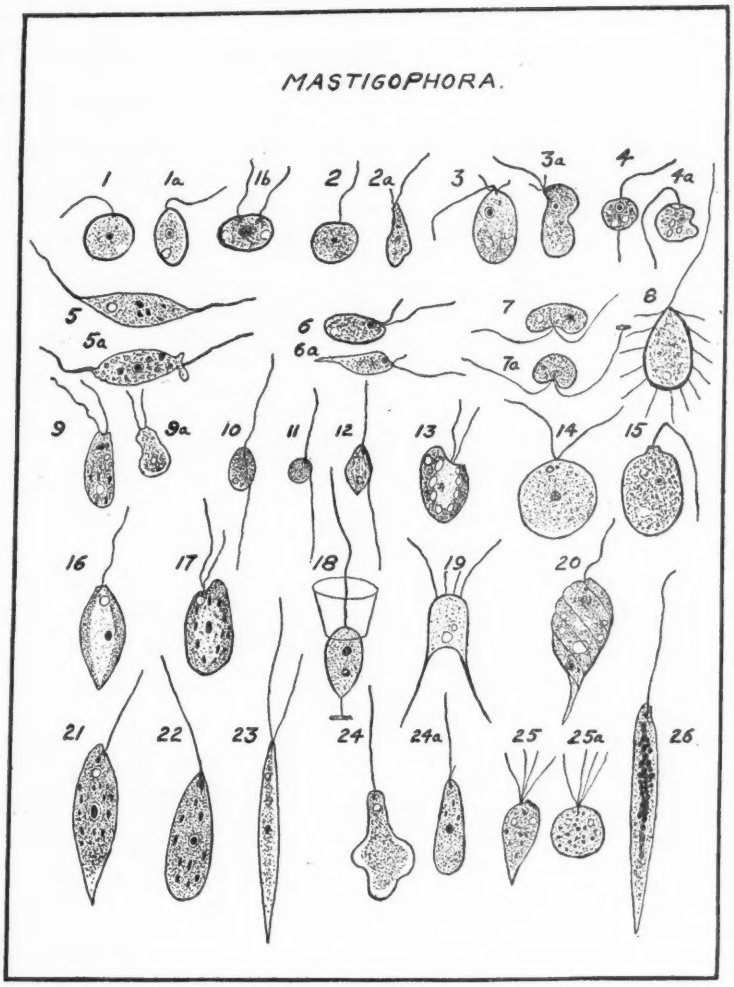

MASTIGOPHORA.
1
1a
1b
2
2a
3
3a
4
4a
5
5a
6
6a
7
7a
8
9
9a
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
24a
25
25a
26

PLATE 3

CILIATA

1. *Holophrya*, sp. (?) Conn. 30–50 μ (38); a variable genus of many species several of which are common in the soil.

2. *Urotricha*, C. & L. sp. Edm. fig. no. 91. 35–50 μ (40); occasional; shows posterior springing hair and lip-like extensions of apical oral aperture.

3. *Enchelys farcimen* Ehrbg. 30–60 μ (40); very abundant; this is one of several similar organisms referred to this genus.

4. *Lionotus fasciola* Ehrbg. 90–120 μ (110); occasional; 2 spherical nuclei; oral aperture located ventrally but usually not visible.

5. *Prorodon teres* (?), Ehrbg. 60–150 μ (75); occasional; oral opening terminal; nucleus central; the usually described species is larger than this one.

6. *Dileptus gigas* C. & L. 200–700 μ (400); occasional; body elongate with narrow, flexible neck-like extension anteriorally; nucleus moniliform; contractile vacuoles numerous.

7. *Chilidon cucullulus* Müll. 70–150 μ (110); occasional; oral aperture located in central part of body; contractile vesicles numerous.

8. *Trichoda pura* Ehrbg. 30–45 μ (35); occasional; endoplasm very transparent showing central oval nucleus; movements very rapid.

9. *Glaucoma scintillans* Ehrbg. 40–100 μ (55); common; oral aperture near anterior extremity with small vibrating membrane; movements very rapid.

10 *Loxophyllum flexilis* Stokes. 70–150 μ (115); rare; nucleus moniliform; body anteriorly pointed.

11. *Uronema marina* Duj. 22–40 μ (30); occasional; body ovate with a single posterior seta; oral aperture ventral with small membrane.

12. *Colpidium striatum* Stokes. 45–75 μ (60); abundant; nucleus central; a posterior contractile vacuole; pharynx ventral with small undulating membrane.

13. *Colpoda cucullus* Stein. 30–50 μ (35); extremely abundant; nucleus central; contractile vacuole posteriorly located; distinct groove ventrally.

14. *Colpoda saprophila* Stokes. 25–40 μ (30); abundant; ventral border anteriorly notched; recurved pharnyx very conspicuous; wide ventral groove. (This species is designated as "*Tillina saprophila*" by Stokes.)

15. *Metopus sigmoides* Clap. & Lach. 90–160 μ (120); occasional; body very contractile; contractile vacuole terminal; several long terminal setae.

16. *Paramoecium trichium* Stokes. 65–100 μ (85); rare; broad oral groove; double contractile vacuole at anterior extremity; nucleus ovai and centrally located.

17. *Blepharisma* (*Apgaria*) *ovata* (?) Stokes. 80–150 μ (100); rare; body much flattened; anterior end acute; membrane covering lower part of peristome sometimes visible.

18. *Cyclidium glaucoma* Ehrbg. 18–30 μ (22); very abundant; body covered with long rigid cilia; hood-like membrane covers oral aperture; movements are darting and eccentric in nature.

19. *Pleuronema chrysalis* Ehrbg. (Stein). 30–70 μ (45); occasional; body curved; a prominent undulating membrane extends from large the furrow-like peristome.

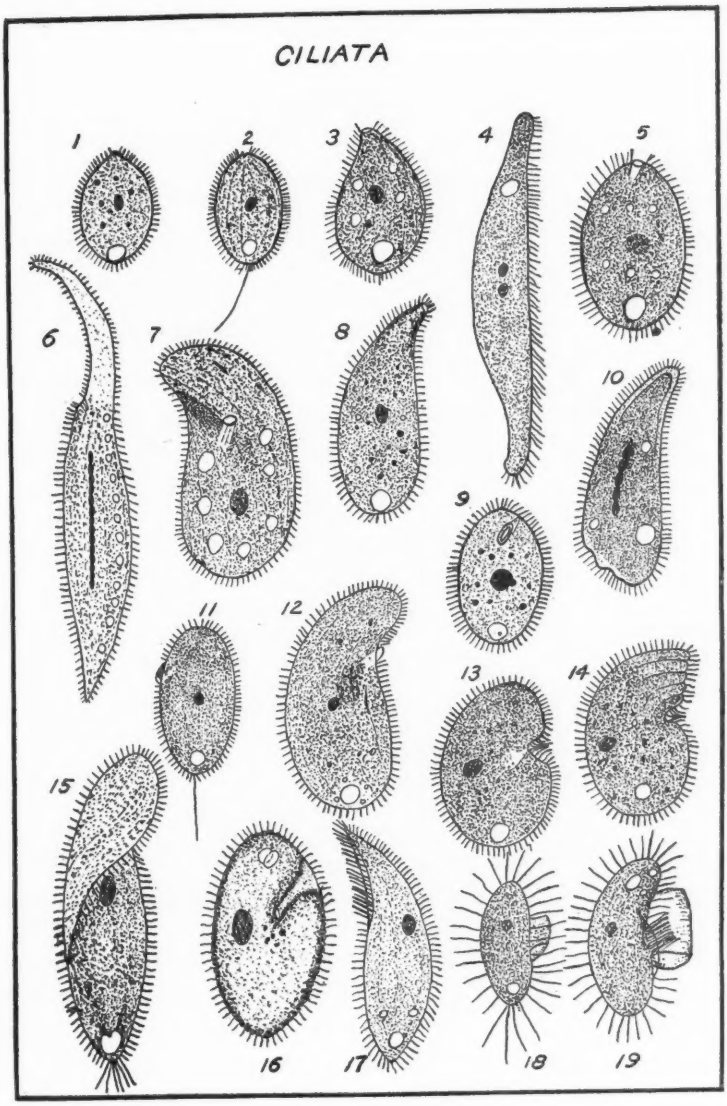

CILIATA
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

PLATE 4

CILIATA (CONTINUED)

1. *Halteria grandinella* O. F. Müll. 25–35 μ (30); common; setae fine and long; movements springing and erratic.

2. *Strombidium*, Clap. & Lach. sp. Conn (?). 30–60 μ (40); rare; like *halteria* but has no bristles; movements very rapid and erratic.

3. *Oxytrichia pelionella* Müll. 70–100 μ (80); common; 2 oval nuclei; 2 anterior and 5 anal styles.

4. *Uroleptus dispar* (?) Stokes. 125–200 μ (150); occasional; body elongate, oblanceolate and elastic; double ovate nucleus; 3 frontal and no posterior styles.

5. *Stylonichia mytilis* Ehrbg. 100–200 μ (135); common; (ventral view); 8 frontal, 5 ventral and 5 anal styles and 3 posteriorly placed setae.

6. *Euplotes charon* Müll. Ehrbg. 70–100 μ (80); occasional; (dorsal view); 7 frontal and 3 ventral styles; nucleus ribbon-like.

7. *Aspidisca costata* Duj. 30–50 μ (35); occasional; nucleus curved and ribbon-like.

8. *Vorticella striata* Duj. 35–45 μ (40); common in soil as are many other species of vorticella; striae prominent.

SOIL ALGAE AND DIATOMS

9. *Protococcus viridis* Ag. 3–20 μ; common; color green to red; cells single or in small clusters.

10. *Closterium* sp. (?). 75 μ; occasional; color green.

11. *Nostoc* sp. (?) Cell diam. 4–6 μ; diam. of heterocysts and spores 8–10 μ; occasional; filaments bluish green in color and enclosed in a gelatinous sheath.

12. *Ulothrix*, sp. (?) Cell diam. 6–10 μ; rare; filaments simple and articulate; cell contents green and cell membrane thin.

13. *Botryococcus* sp. (?) 7–14 μ diam.; occasional; cells oval to spherical with thin confluent tegument; masses of cells densely packed forming a botryoid; color light green.

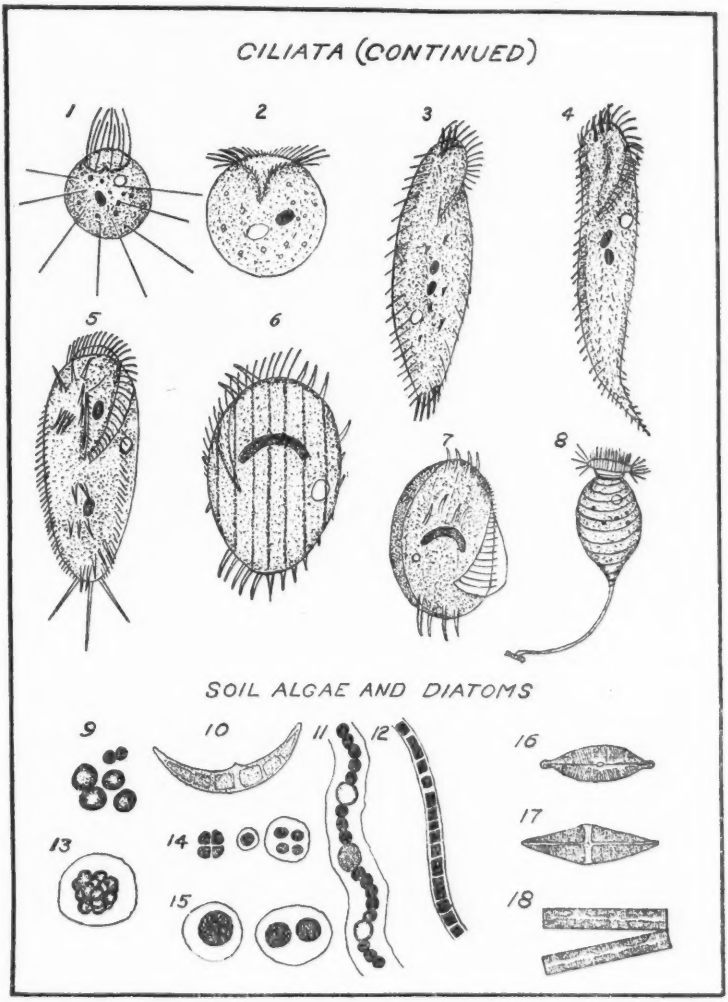
14. *Tetraspora* sp. (?) Cell diam. 4–10 μ; occasional; cells angular to spherical; usually arranged in tetrads; gelatinous sheath sometimes present.

15. *Chroöcoccus cohoerens* (?) Naeg.; 3–6 μ diam.; occasional; solitary or in small groups; gelatinous sheath usually present.

16. *Navicula rhyncocephara* (?) 40 μ; occasional; valves symmetrical and boat shaped; raphé straight with conspicuous medial nodule.

17. *Stauroneis*, sp. (?) 45 μ; occasional; valves symmetrical; there is a clear area along the raphé which also extends up transversely from the medial nodule giving the appearance of a cross.

18. *Diatoma vulgare* (girdle view); 45 μ; rare; frustules attached by their angles forming zigzag chains; values elliptical-lanceolate and lined with fine striae; there is a longitudinal pseudo-raphé.


CILIATA (CONTINUED)
1
2
3
4
5
6
7
8
SOIL ALGAE AND DIATOMS
9
10
11
12
16
13
14
17
15
18

THE REACTION OF THE SOIL AS INFLUENCED BY THE DECOMPOSITION OF GREEN MANURES[1]

L. P. HOWARD

Rhode Island Agricultural Experiment Station

Received for publication December 10, 1919

INTRODUCTION

A common idea associated with the practice of green manuring is that the soil tends to grow more acid. This idea is a result, perhaps, of the ancient and still existing controversy relative to the existence of the so-called humic acids, and to a belief that these acids result from a decomposition of organic residues from green manures or other sources.

Temple (14) has raised the question as to what the effect of green manures is on soil acidity. From his work on nitrification after the addition of large amounts of organic acids, he concludes that injury due to the accumulation of organic acids in arable soils is very doubtful.

Hall (6) cited work at Rothamsted to show that the per cent of calcium in the first 9 inches of soil decreased where an annual application of 14 tons of manure per acre had been made.

Bear and Salter (2) showed that the lime requirement of manure plats became less than that of the check plats.

Hill (8) cited work of Laurent indicating that the harmful effects of green manure result from an accumulation of organic acids.

Ames and Schollenberger (1) have shown by pot experiments, conducted with soil taken from an uncultivated field, that the application of legumes and non-legumes in either dry or green state, has decreased the lime requirement as indicated by the Hopkins method. The dry application was more effective than the green.

White (15) found that when green manures and soil were kept in jars the lime requirements were less than with the soil alone at the end of the first fortnight, and more at the end of the second fortnight.

This brief review of the literature serves to indicate that the quantitative evidence for or against this question is very limited.

At the Rhode Island station there are plats, which, by their similarity of treatment in the past, offer opportunity for such a study and it is the purpose of this paper to present such information as has been secured.

[1] Contribution 265 from the Agricultural Experiment Station of the Rhode Island State College.

HISTORY OF THE FIELD EXPERIMENT

On an acre of land, corn has been grown continuously since 1894 with uniform applications of only chemical manures as a source of plant-food. After a few years the plan of the experiment was modified so that a comparison between a legume and a non-legume, as a means of maintaining the soil humus, might be made. At the time of the last cultivation of the corn, each season, one-quarter was for years usually sown with crimson clover, while another quarter was sown to rye. The intermediate quarter served as a check plat. This practice was followed unchanged until recent years when the legume seeding was modified to include other legumes beside the crimson clover. Lime was added in 1899 at the rate of one ton of slaked lime, in 1911 at the rate of 1500 pounds of calcium oxide in slaked lime, and in 1914 at the rate of 1 ton of ground limestone (7).

FACTORS INFLUENCING THE REACTION

In the following paragraphs are considered the factors which operate on the various plats to influence the reaction of the soil.

A. The removal of soluble bases and acids by leaching. This depends mainly upon seasonal conditions. The leaching from the check plat may serve as an index of the magnitude of this factor, except as influenced by the cover crops.

B. The removal of acids and bases by cropping. The sizes of the crops removed during the first years of the experiment were not markedly different. The difference has become accentuated in later years.

The following statement shows the amounts of corn and stover removed from a quarter acre from 1897 to 1914:

	LEGUME PLAT	CHECK PLAT	RYE PLAT
Ear corn (bushels)	210.1	153.9	163.5
Stover (tons)	8.02	6.61	7.32

We may well ask the question as to the amounts of acids and bases removed by cropping, and what the variation has been in the various plats. The calculations are based upon the following estimated analysis of field-cured corn:

	EAR CORN	CORN STOVER
	per cent	*per cent*
Nitrogen	1.1	0.8
Phosphoric acid	0.6	0.4
Sulfuric oxide	0.3	0.2
Chlorine	0.1	0.5
Potassium oxide	0.4	1.3
Calcium oxide	0.2	0.5
Magnesium oxide	0.2	0.3

The acids are calculated from the nitrogen, phosphorus, sulfur and chlorine removed, and the bases from the potassium, calcium and magnesium. These are the most important elements in a consideration of the acids and bases. It is assumed that the nitrogen entered the plant from the soil fully oxidized, and that each atom of phosphorus was equivalent to an atom of calcium. Silicon is omitted because its reaction is so mild. The calculations show that an excess of acidic over basic ions was removed. The relation is brought out by the following:

Removal by corn, equivalent to pounds of calcium oxide

	ACIDS REMOVED	BASES REMOVED	EXCESS ACIDS OVER BASES REMOVED
1 bushel ear corn (70 pounds)	2.075	0.502	1.573
1 ton stover	49.04	33.82	15.22

Total excess acids over bases removed per quarter acre, 1897 to 1914

	LEGUME PLAT	CHECK PLAT	RYE PLAT
By ear corn	331	242	257
By stover	122	101	111
Total	453	343	368

These figures serve to indicate the amounts of soluble salts which were removed, and the relation of acids to bases. The difference between the check and other plats tends toward a conservation of soil bases and in reality, a reduction in the acidity. The reduction due to this consideration should be more marked on the legume area.

C. Addition of fertilizers. The addition has been the same on the three areas, except that beginning with 1915 the legume plat has received a smaller amount of nitrogen.

D. Decomposition of organic matter. Any difference in the plats may be attributed to this factor; that is, the fluctuations in the reaction on a given plat will be the resultant of the factors ($A + B + C + D$) which are being considered; A, B and C being factors operating about the same on the different plats while the factor D, due to the decomposition of organic matter, is the variable on which differences in the reaction of the legume and rye plats may be conceived to depend.

PLAN OF THE PRESENT EXPERIMENT

The plan as followed consisted in sampling the soils at various periods during the season to follow changes in acidity, and likewise in organic matter. It also seemed desirable to supplement the changes in the field with those which would occur in the laboratory.

Soil samples and treatment. Twelve borings 12 inches deep were taken from each plat with a soil sampler made of 2-inch pipe. The samples were brought to the laboratory and weighed, partially air-dried, mixed, sub-sampled and passed through a 2-mm. sieve. The determinations were made immediately on the partially air-dried soil. Moisture determinations were made and all analyses recorded on dry soil finer than 2 mm. Some of the soil was dried more thoroughly, and stored in sealed jars for work done subsequently.

Loss on ignition. The soil was dried at 100°C. for 24 hours and then ignited to constant weight. This loss on ignition includes, besides organic matter, especially water of hydration. It is believed, however, that on these soils the differences are largely attributable to the organic matter.

Reaction of the soil. (a) As indicated by lime-requirement methods. During the season of 1915 the MacIntire (13) and Jones (11) methods were used. In 1916 the ammonia method was used. Later the requirements of the stored samples were determined also by the ammonia method (10). (b) As measured by the concentration of hydrogen ions. In this work the colorimetric procedure of Gillespie was followed, using the sulfon ephthalein indicators (5) and phthalate buffer solutions of Lubs and Clark (3). These determinations were made only on the stored soil samples.

WORK ON THE FRESH SAMPLES TAKEN IN THE FIELD IN 1915

At the time of plowing for the corn in the spring of 1915 (May 18), the legume plat was well covered with vetch but the crimson clover was almost entirely winter-killed; the non-legume plat was well covered with rye. The average height of the rye at the date of plowing was 3 feet. The weight of the green crop from 1 square yard was 3.7 pounds of the legume and 1.3 pounds of the rye.

Soil samples were taken at frequent intervals from the date of plowing, and the lime requirements measured by the Jones and MacIntire methods. The fluctuations as indicated by these methods are recorded in table 1.

In table 1 it is seen that with the MacIntire method the requirement of the check plat increases slightly around the first of August and then diminishes so that the initial and final acidities are not very different. On the rye area the same mid-season increase takes place but the decrease in the latter part of the season is more marked and finally becomes quite decided. In opposition to the other two plats, the legume plat shows a decrease on August 13. This was followed by an increase so that the determinations compare fairly well in the fall with those recorded early in the season.

The Jones method detected only a slight variation, from which no positive statements are justified.

It has been shown in a former publication (9) that under closely regulated conditions the MacIntire method, as modified, would yield requirements in which the probable error of a given determination was 62.6 pounds CaO per

2,000,000 pounds of soil. With the Jones method, duplicate requirements varied from each other by 150 pounds CaO per 2,000,000 pounds of soil. By the ammonia method a number of determinations showed that the probable error could be reduced to 90 pounds of CaO per 2,000,000 pounds of soil.

The error in sampling the soil is shown by the fact that the range in the requirement of five samples taken from the legume plat on September 21, 1915, as measured by the MacIntire method, was 22 pounds CaO per 2,000,000 pounds of soil or well within the limits of error of the method itself.

TABLE 1

CaO *requirement per 3,000,000 pounds soil finer than 2 mm.*

DATE OF SAMPLING	MACINTIRE METHOD			JONES METHOD		
	Check	Rye	Legume	Check	Rye	Legume
1915	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
June 4	3276	3092	4175		1263	1620
June 10		3276	4242		1350	1738
June 21		3335	3966	1296	1350	1620
June 30	3393	3066	4301	1188	1361	1512
July 10	3150	3528	4536	1296	1296	1944
July 31	3486	2889	4578			
August 13	3612	3864	3270			
August 27	3237	2721	3723			
September 9	3360	1981	4253			
September 21			4091			

WORK ON THE FRESH SAMPLES TAKEN IN THE FIELD IN 1916

The crops on the legume plat were largely winter-killed; the alfalfa and sweet clover of the mixture were alive but had produced only a small growth. Fluctuations occurring on this plat, therefore, depended largely upon organic residues which had accumulated during the previous 20 years.

The non-legume plat was well covered with rye but the size of the crop was below normal. The average height was 2 feet. The green weight of rye, cut from 1 square yard just before plowing on May 18, was 1.4 pounds.

It was noticed in 1916 during the process of sampling that although the check and rye areas appeared quite similar and uniform, more variations existed on the legume plat. This plat slopes gently to the north and the variation in depth of surface soil is from 6.5 inches on the south to 9 inches on the north. The average depth of the surface soil of the three sections was:

	inches
Check	6.0
Rye	7.75
Legume	7.0

The weight of 1 cubic foot of soil, on May 9, 1916, was:

	pounds
Check	103.0
Rye	105.4
Legume	106.2

The weight of 15 borings, 12 inches deep (as sampled) was:

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*lbs.*	*lbs.*	*lbs.*
April 25	31.0	29.5	28.0
May 2	30.5	29.5	27.8
May 9	31.0	29.0	29.0*
May 31	27.5	26.8	26.0

* 22.5 per cent moisture.

Table 3 shows during the field experiment an increase, or less decrease, in organic matter due to the cover crops, and that the tendency was toward a progressive decrease in organic matter from the date of plowing to the end of the season. If the averages are accepted as a criterion, it may be said that during the field experiment, the rye cover crops had increased the per cent of organic matter in the soil by 0.17, and the legume cover crops by 0.70.

TABLE 2

Variation in the per cent of soil finer than 2 mm.

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*per cent*	*per cent*	*per cent*
April 13	92.4	92.9	92.5
April 25	96.0	96.5	95.9
May 2	92.5	96.0	94.1
May 9	93.1	96.9	96.7
May 31	93.3	95.9	95.2
June 5	93.2	95.1	96.7
June 14	92.8	94.1	97.1
June 20	94.2	95.6	97.1

TABLE 3

Differences in organic matter as indicated by the per cent of loss on ignition

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*per cent*	*per cent*	*per cent*
April 13	4.31	4.52	5.25
May 18	4.46	4.56	5.04
May 31	4.42	4.62	5.20
June 5	4.37	4.64	5.00
June 14	4.40	4.57	5.02
June 20	4.43	4.53	5.10
July 7	4.33	4.49	4.95
August 24	4.25	4.42	4.97
Average	4.37	4.54	5.07

TABLE 4

Fluctuations in the acidity of the field samples soon after sampling, as determined by the ammonia method

DATE OF SAMPLING	REQUIREMENTS OF CaO PER 3,000,000 POUNDS OF SOIL		
	Check plat	Rye plat	Legume plat
1916	*lbs.*	*lbs.*	*lbs.*
April 13	3280	3140	5128
April 25	2954	2854	4266
May 2	3515	3603	5249
May 9	3649	3654	4924
May 18*	3295	3040	3814
May 31	3745	3866	5758
June 5†	3442	3247	4985
June 14	2954	2856	4435
June 20	3072	3073	
July 7	3226	3286	4423
August 24	3269	2994	4433

NOTE: The amounts recorded represent the average of triplicate determinations.

* Just before plowing.

† On this date the soil contained a large amount of ammoniacal nitrogen as indicated by the blank determination. It was then equivalent to an average lime requirement of 669 pounds. With the exception of May 31, other blanks were negligible.

DECOMPOSITION IN LABORATORY-TREATED SOILS IN 1916

The decomposition under field conditions is associated with a great many factors which are extremely difficult to estimate. Inasmuch as the size of the green crops returned to the soil was below normal in the spring of 1916, it seemed desirable to supplement the field studies with laboratory studies carried on at the same time, so that the application of green material could be increased and thereby accentuate any changes which might occur.

Accordingly, 102 borings 12 inches deep were taken from the check plat. The soil and subsoil were kept separate. Each of three Wiley pots, 12 inches in diameter and depth, was filled beneath with 26 pounds of subsoil and above with a layer of 35 pounds of surface soil.

One pot received 634 gm. of finely-cut, fresh red clover, of which 135 gm. were roots and 499 gm. were tops. Another pot received the same amount of rye in which the ratio of roots to tops was 3 to 5. The third pot received no organic matter. The mixtures were kept thoroughly stirred and a moisture content of 25 per cent was maintained.

The figures in table 5 indicate that during the period studied, rye produced an acid condition equivalent to about 500 pounds CaO per acre, over that indicated by the check pot. The pot receiving clover showed a slightly increased acidity but not so marked as that indicated by rye. Ammonification

proceeded in the case of each green manure. The percentage of organic matter as indicated by loss on ignition was materially increased. The decomposition of clover proceeded more rapidly than that of rye.

TABLE 5

Fluctuations in the loss on ignition and in the acidity of the pot samples soon after sampling

DATE	TREATMENT	LOSS ON IGNITION	BLANK NH_3 IN 25 GM. SOIL	NH_3 RETAINED BY 25 GM. SOIL — BLANK	CaO PER 3,000,000 POUNDS SOIL
1916		*per cent*	*mgm.*	*mgm.*	*lbs.*
May 25	Original soil	4.80	0.05	14.42	2848
July 25	Check	4.81	0.68	14.62	2888
	Rye	5.70	0.68	16.25	3210
	Clover	5.50	0.68	15.44	3050
September 12	Check	4.83	0.15	15.02	2967
	Rye	5.40	1.00	17.45	3447
	Clover	5.18	1.80	16.04	3168
December 16	Check	4.79	0.07	15.23	3008
	Rye	5.20	0.54	17.20	3397
	Clover	5.01	0.41	15.91	3143

SOIL CHANGES IN 1915 AND 1916 AS EXHIBITED BY THE DRIED STORED SAMPLES

This work on the samples collected over a period of two years shows that variations are maintained in the dried soils, as manifested in their ability to retain different amounts of ammonia. In the 1915 samples a difference existed between the initial and final acidity of the check as compared with those of the plats receiving organic matter, which relatively would credit the rye with increasing the requirement equivalent 637 pounds of CaO, while the increase due to the legume would be 942 pounds of CaO.

During the 1916 season the amount of green manure added in legumes was so slight that any variation in that year must be attributed to the addition of organic matter in former years. The rye plat exhibited a net increase of 65 pounds of CaO, while the legume plat recorded a net decrease of 236 pounds of CaO. Both quantities have little significance, inasmuch as they approach the error limits of the method, and because of fluctuations between near periods of sampling.

The net result over the period of 2 years indicates a decreased acidity except in the legume plat. The net difference between check and non-legume as compared with that between check and legume, suggests that the rye caused an increased requirement of 33 pounds while the legume caused an increase of 370. As has been stated, the last application of lime was made in the spring of 1914 in 1 ton of finely ground limestone per acre. This fact may have some significance when considering net changes in lime requirement even during 1915 and 1916.

TABLE 6

Fluctuations in acidity in soils stored over a period of 2 years, as indicated by the ammonia method

DATE OF SAMPLING	REQUIREMENT OF CaO PER 3,000,000 POUNDS OF SOIL FINER THAN 2 MM.		
	Check plat	Rye plat	Legume plat
1915	*lbs.*	*lbs.*	*lbs.*
June 4	2686	2518	3559
June 10		2988	3962
June 21	2686	2821	3559
June 30	2250	2921	3727
July 10	2418	3056	3761
July 31	2250	2753	3559
August 13	2686	4063	4101
August 27	2418	2586	3559
September 21	1914	2383	3729
1916			
April 13	2383	2183	3862
April 25	2047	2418	3358
May 2	2317	2686	3761
May 9	2250	2686	3425
May 19	2383		3358
May 31	2921		
June 5		2686	
June 14	2921	2686	4154
June 20	2388	2753	3559
July 7		2988	3727
August 24	2518	2383	3761

TABLE 7

Seasonal variation in the CaO *requirement and the relation of final to initial requirement, as given in table 6*

SEASON	FLUCTUATION IN CaO REQUIREMENT			FINAL, AS COMPARED WITH INITIAL CaO REQUIREMENT		
	Check	Rye	Legume	Check	Rye	Legume
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
June 4 to September 21, 1915	772	1680	600	−772	−135	170
September 21, 1915, to April 13, 1916				469	−200	133
April 13 to August 24, 1916	874	805	796	135	200	−101
June 4, 1915 to August 24, 1916				−168	−135	202

THE HYDROGEN-ION CONCENTRATION IN THE SOILS

It is to be regretted that the true reaction of the soil solution, as measured by the hydrogen-ion concentration, has not received more attention. The question may well be raised, however, as to the relation which the hydrogen-ion concentration, measured by making an aqueous extract in colorimetric work, and a suspension in electrometric work, bear to the concentration of hydrogen ions in the true soil solution.

Whatever the relation may be, it is obvious that if a high concentration of hydrogen ions is recorded in the aqueous extract, the concentration will be as great or greater in the true soil solution with which the plant is associated.

TABLE 8

Hydrogen-ion concentration in the soils after storage

DATE SAMPLED	SOIL	pH	H'
	Field soils		
1915			
June 4	Check	6.0	1.0×10^{-6}
July 10	Check	6.0	1.0×10^{-6}
August 13	Check	6.0	1.0×10^{-6}
September 9	Check	6.0	1.0×10^{-6}
June 4	Rye	6.1	0.8×10^{-6}
August 13	Rye	5.8	1.6×10^{-6}
September 9	Rye	6.4	0.4×10^{-6}
June 4	Legume	6.0	1.0×10^{-6}
July 31	Legume	5.9	1.2×10^{-6}
August 13	Legume	6.0	1.0×10^{-6}
September 21	Legume	6.0	1.0×10^{-6}
1916			
April 25	Check	6.2	0.6×10^{-6}
May 31	Check	6.1	0.8×10^{-6}
June 14	Check	5.9	1.2×10^{-6}
August 24	Check	6.1	0.8×10^{-6}
April 13	Rye	6.3	0.5×10^{-6}
June 14	Rye	5.9	1.2×10^{-6}
July 7	Rye	6.0	1.0×10^{-6}
August 24	Rye	6.2	0.6×10^{-6}
May 19	Legume	5.8	1.6×10^{-6}
June 5	Legume	5.8	1.6×10^{-6}
June 14	Legume	5.9	1.2×10^{-6}
August 24	Legume	6.0	1.0×10^{-6}
	Pot-treated soils		
1916			
December	Check	5.9	$1.2 - 10^{-6}$
	Rye	5.9	$1.2 - 10^{-6}$
	Legume	5.9	$1.2 - 10^{-6}$

The results here recorded in table 8 are with the dried soils, and consequently an acidity due to the easily volatile acids, which might have been measured in the moist soil at the time of sampling, does not enter into the reaction.

It is seen that the concentration of hydrogen ions from the three plats sampled in 1915 shows variations in pH of from 5.8 to 6.4. The greatest variation occurs in samples from the rye plat, the other two plats remaining more constant. It is significant that with the rye plat the variations in concentration of hydrogen ions, follow in a general way the fluctuations in the lime requirement.

In 1916 a midsummer increase as compared with final acidity is seen in each soil, and the legume plat was the only one to show a higher initial than final acidity.

The final reaction of pot-treated soils appears to have been unaltered by the addition of organic matter.

GENERAL DISCUSSION

In the absence of records of soil temperatures and soil moisture, the mean air temperature and the precipitation may be considered briefly in connection with the acidity fluctuations in the field soil, relying chiefly on the determinations obtained with the fresh soil. In 1915, 8 days prior to taking the first sample on June 4, there had been no rain and an average mean temperature of 48°F. June had an average mean temperature of 59° and a precipitation of 1.36 inches, accompanied by no marked changes in acidity. During the first 9 days in July more rain fell than during all of June but the mean temperature had not changed much. A small increase in acidity was general. There was only 0.72 inch of rainfall during the remainder of July, and on the thirty-first, as a rule, no marked change in acidity was recorded. In the first 12 days of August, 5.2 inches of rain fell, accompanied by a mean temperature of 63°. On August 13, with the exception of the legume plat, which had already reached the maximum, the highest acidity was recorded, and by the twenty-seventh the tendency was generally downward so that in no case was there a greater acidity at the close of the season, which was dry, than at the beginning.

In 1916, the sampling was begun much earlier than in 1915, namely, on April 13, as soon as the frost was out of the soil. There was a fair prospect with the rye, but the legumes were practically a failure. The lime requirement of the legume plat proved to be about a ton of calcium oxide greater than that of the other two plats. During the succeeding 12 days, 2.6 inches of rain fell and the acidity dropped a few hundred pounds, which may have been due to leaching. Although during the following 7 days 1.7 inches of rain fell, the previous drop in acidity had been fully offset, as is shown by the determinations of May 5 and 9. There were 4.6 inches of rainfall during the remainder of the month, over an inch of which fell 2 days before the May 31 samples were taken. The highest acidity of the summer had then been reached; whereas, the lowest acidity and highest ammonia formation was recorded on June 5, about another inch of rain having fallen in the meantime. The temperature between May 24 and June 4 was fairly uniform, with a mean of 57°. The plats were plowed

May 18, fertilized May 23, and planted May 25. Samples of soil were also collected on June 14 and 20 and July 7, but only a moderate increase in acidity was revealed by their examination. Over 6 inches of rain had fallen during the period since June 4, and heavy rainfall continued throughout the remainder of July. The driest period of the summer was during the last half of August. The mean temperature in August was 65°. One more lot of samples was taken on August 20 but not much change in acidity was registered. There had been no net increase in acidity over that shown on April 13. Below are given the average of the mean daily temperatures, and the rainfall in inches during the warmest four months.

		MAY	JUNE	JULY	AUGUST
Mean temperature (°)	1915	50.5	59.4	65.1	63.7
	1916	51.0	56.7	64.0	65.0
Precipitation (in.)	1915	2.64	1.36	2.28	7.74
	1916	4.80	5.77	11.75	2.77

In 1915 the highest acidity was in the August 13 samples; and in 1916 it was in the May 31 samples.

No conclusions seem to be warranted from the limited data at hand regarding the seasonal fluctuations. Possibly a liberal rainfall but not enough to cause a leaching of acid substance from the surface foot of soil is conducive to high acidity (4).

If the determinations on the stored samples of the two years, which were analyzed by a uniform method, are used as a criterion, it may be seen that there was practically no net increase in acidity during the years 1915 and 1916, finely ground limestone having been applied in 1914. In fact, there is no evidence that any acidity has resulted from the use of rye as a cover crop for a quarter of a century. The legumes, however, have during the same time, considerably increased the lime requirements.

In the uncropped soils treated in the laboratory with an equal weight of green rye as compared with clover, the rye increased the lime requirement 300 to 400 pounds, or about twice as much as the clover, and the increase was maintained from July to December.

It is evident that the results of the work on samples collected during 1915 and 1916, taken together, were scarcely an index of the net cumulative effect exerted by the cover crops through the longer period of time.

REFERENCES

(1) AMES, J. W., AND SCHOLLENBERGER, C. J. 1916 Liming and lime requirement of soil. Ohio Agr. Exp. Sta. Bul. 306.

(2) BEAR, F. E., AND SALTER, R. M. 1916 The residual effects of fertilizers. W. Va. Agr. Exp. Sta. Bul. 160.

(3) CLARK, W. M., AND LUBS, H. A. 1916 Hydrogen electrode potentials of phthalate, phosphate and borate buffer mixtures. *In* Jour. Biol. Chem., v. 25, p. 479–510.

(4) CONNER, S. D. 1918 Soil acidity as affected by moisture conditions of the soil. *In* Jour. Agr. Res., v. 15, p. 321–329.

(5) GILLESPIE, L. J. 1916 The reaction of soil and measurement of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 1, p. 7–16.

(6) HALL, A. D., AND MILLER, N. H. J. 1905 Effect of plant growth and of manures upon the retention of bases by the soil. *In* Proc. Roy. Soc. London, v. 13, p. 1–32.

(7) HARTWELL, B. L., AND DAMON, S. C. 1918 Miscellaneous experiments with corn. R. I. Agr. Exp. Sta. Bul. 173, p. 18–27.

(8) HILL, H. H. 1913 The effect of green manuring on soil nitrates under greenhouse conditions. Va. Agr. Exp. Sta. Tech. Bul. 6.

(9) HOWARD, L. P. 1917 Determination of the lime requirements of soils by the use of calcium carbonate. *In* Jour. Assoc. Off. Agr. Chem., v. 3, p. 141–144.

(10) HOWARD, L. P. 1918 The relation of the lime requirements of soils to their retention of ammonia. *In* Soil Sci., v. 6, p. 405–411.

(11) JONES, C. H. 1913 Method of determining the lime requirement of soils. *In* Amer. Fert., v. 38, no. 11, p. 29.

(12) KOBER, P. A., AND GRAVES, S. S. 1913 Quantitative ammonia distillation by aeration, for Kjeldahl, urea and other nitrogen estimations. *In* Jour. Amer. Chem. Soc., v. 35, p. 1597.

(13) MACINTIRE, W. H. 1915 Method for Determination of immediate lime requirements of soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 864.

(14) TEMPLE, J. C. 1914 Nitrification in acid or non-basic soils. Ga. Agr. Exp. Sta. Bul. 103.

(15) WHITE, J. W. 1915 Soil acidity: the relation of green manures to its development. *In* Penn. State Coll. Ann. Rpt., 1914–15, pt. 2, p. 60–86.

SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF CHLORIDES IN SOIL

C. T. HIRST AND J. E. GREAVES

Department of Chemistry, Utah Experiment Station

Received for publication December 30, 1919

The advancements made in any field of endeavor are limited by the rapidity with which methods are developed in that subject. The value of the results in any experiment is directly proportional to the accuracy of the method used. It is useless to plan and conduct long and costly experiments when the accuracy of the analytic method is questionable or unknown. Hence, one need offer no apology for work, the object of which is the perfection of new or the study of old analytical methods. Moreover, methods have often been devised and tested for use on one substance or under one set of conditions and may or may not give good results when used on other substances and under other conditions.

Methods have been devised for the determination of chlorine in water, milk, blood, urine, soil and other naturally-occurring substances. No two of the methods give equally reliable results under all conditions. Hence, this investigation was designed to study and select the one best suited for use in the determination of chlorides in soil.

An ideal method for the determination of chlorides in soil must be accurate when applied to substances containing little or much chlorine. It must be accurate when used on soils having large quantities of other soluble salts and those containing large amounts of organic matter. It should be fairly easy of manipulation and of such a nature that a great number of determinations can be made in a short time.

The gravimetric method is not suitable for the determination of chlorides in soil on account of the time it consumes where so many determinations are to be made. Moreover, waters and solutions which contain very small amounts of chlorides do not form coagulated precipitates unless first concentrated by evaporation. Certain substances have a solvent action on silver chloride when heated, among them being the nitrates of the alkali and alkali earth metals (5, p. 199) and silver nitrate in strong solution. Moreover, heating the chloride solution with nitric acid before the addition of the precipitating agent, as is usually done, results in a loss of chlorides (5, p. 522).

In the determination of alkali or alkali earth chlorides volumetrically by Mohr's method, the solution must be absolutely free from acid or any great excess of alkali. It is best to have them neutral and cold (13). Yoder (8)

states that there should be no carbonates or phosphates present, as the silver salts of these acids are less soluble than the chromate, and the solution should have a certain definite concentration in order to give the most definite color change. If the chlorine content is below 7.5 parts per million the solution should be concentrated (14). Kolthoff (7) found that 0.7 to 1 cc. of 1 *N* potassium chromate per 100-cc. sample was the best amount of indicator to use and that the H-ion concentration could vary between 5×10^{-7} and 5×10^{-11}. He found also that the harmful effects of phosphates can be avoided by using more indicator, that borax, SO_4 and NH_3 below 50 mgm. per liter are harmless, and that proteins and iron salts must be absent. Winkler (17) states that a comparison should be made by running duplicate samples. To one silver nitrate is added until a faint red color is produced which is then just discharged with sodium chloride. To the other silver nitrate is added until a faint red color is formed and persists for five minutes. A correction is subtracted from the burette reading, the amount of the correction increasing with the amount of silver nitrate used. Kolthoff (7) denies that the correction increases with the amount of chlorine, as claimed by Winkler.

Tillman and Heublein (14) claim the Mohr method gave too high results when too little indicator is used, the titration being carried too far. If sufficient indicator is used no correction is necessary. They state that at least 1 cc. of a 10 per cent solution of potassium chromate should be used for each 100 cc. of solution.

An error may be introduced by a rise in temperature since the solubility of silver chromate increases with a rise in temperature (16). The presence of nitrates also increases the solubility of silver chromate.

For small amounts of chlorine in concentrated solutions the Mohr method gives very definite results. If, however, the volume of the solution is too large the results are not very accurate. In all cases the sensitiveness of the indicator should be tested by determining the quantity of silver solution necessary to produce the red shale used in the titration when no chlorides are present.

When a decinormal solution of silver nitrate is used an excess of about 0.2 cc. is required and this must be subtracted from the amount used. The color change is sharper when sodium arsenate is used as indicator and no correction is then necessary (8). Stuart (12) found the Mohr method could not be used on peaty waters.

In the original Volhard method the excess of silver nitrate was titrated with the alkali thiocyanate in the presence of precipitated silver chloride without any special treatment. This gives results which are satisfactory with large quantities of chlorides, but which are too high with small quantities (15). Drechsel (4) shows this to be due to the silver thiocyanate, being less soluble than the silver chloride, so that on stirring the red ferric thiocyanate gradually decomposes, forming silver thiocyanate and making it difficult to obtain the true end-point, since the red color becomes permanent

only after considerable excess of thiocyanate has been added. He overcame this difficulty by adding an excess of silver nitrate to the chloride solution contained in a graduated flask and shaking until the silver chloride coagulated, then filtering through a dry filter, adding the indicator to an aliquot part and titrating the excess of silver nitrate with the thiocyanate solution.

Volhard in his original work contended that the velocity of decomposition of ferric thiocyanate was so slow that it would not affect precise work. But Rosanoff and Hill (9) showed it to be rather high, causing an error in some cases of as much as 2.9 per cent where the silver chloride is left in the solution, whereas when it is removed before the addition of the sulfocyanate the error does not exceed 0.17 per cent.

The Volhard method, without the removal of the silver chloride, is accurate only when there is a rather large amount of chlorides present and when the solution is not too dilute. When the silver chloride has been thoroughly

TABLE 1

Chlorides obtained by the Volhard method when the solution is heated and treated with alcohol or ether

TREATMENT	NUMBER OF DETERMINATIONS	CHLORIDES CALCULATED AS NaCl	PROBABLE ERROR
		per cent	
Heated to boiling	3	4.944	±0.01
10 cc. ether	4	4.937	±0.007
10 cc. ethyl alcohol	4	4.937	±0.007

coagulated by boiling after the addition of an excess of silver nitrate, it reacts less readily with the thiocyanate and it is possible to obtain correct results after the solution has been cooled without its removal (10). Silver chloride may be as well coagulated by the addition if ether as by boiling, and under these conditions the chlorides may be correctly determined by the Volhard method without the removal of the precipitated silver chloride (1). In our work we found that alcohol was just as efficient as the ether or boiling.

The results obtained by us after coagulating the chlorides in soil solution by the various methods are given in table 1.

The differences in the various results are well within the experimental error, hence the ether and the alcohol are just as efficient coagulants as is heat. Moreover, the use of ether or alcohol saves the time used in heating and cooling the solution. For these reasons we have used alcohol as the coagulating agent in our work.

From the preceding brief review of the literature it would appear that the Mohr and Volhard methods are best suited for the determination of chlorides in soil, hence our experimental work has been based on them.

Preparation of a clear filtrate. The first prerequisite in the determination or chlorides in the soil is the preparation of a clear solution. Investigations

have already been carried out in this laboratory showing the relative efficiency of the Pasteur-Chamberland filters, centrifuge, calcium oxide, calcium carbonate, talc, ferric sulfate, potassium alum, sodium alum and iron alum as clarifying agents, and also their effects on the determination of nitric nitrogen in the soil solution (6).

Of these the potassium alum, the Pasteur-Chamberland filter and the centrifuge gave the most satisfactory solutions and interfered the least with the determination of the nitric nitrogen in the solution.

In the present work, therefore, these three methods were used for obtaining the solution in which the chlorides were to be determined. The results obtained by the various methods are given in table 2.

The soil used was a heavy black clay loam collected from a depression about four miles west of Corrinne, Utah. Evaporation had left the soil with a thick incrustation of alkali salts. The soil was dried in the laboratory, pulverized, sieved, and preserved in wooden boxes.

TABLE 2

Chlorides obtained from soil solutions which were clarified by the filter, the centrifuge or alum

TREATMENT	CHLORIDES	ERROR	NUMBER OF DETERMINATIONS
	per cent		
Filter	4.998	±0.011	18
Centrifuge	5.02	±0.013	8
Alum	4.998	±0.01	18

In so far as these results show, there is nothing to choose between the filter and the alum since they give concordant results and the probable error is about the same in each case. The centrifuge, however, gives higher results. It is possible that colloids or proteins from the soil were present and remained in suspension with the centrifuge and would be removed with the alum and filter. It is a fact that the centrifuge at the speed at which we run it, about 1200 revolutions per minute, always gave a murky, cloudy solution, whereas the filter and the alum gave perfectly clear solutions.

It seems, therefore, that the filter or the alum where permissible is the best means for obtaining a clear filtrate. This is in keeping with our finding on the preparation of a clear solution for the determination of nitric nitrogen in the soil solution, where we found that alum and the filter gave clear filtrates and interfered least with the recovery of the nitric nitrogen.

The amount of chlorides in the soil. In order that we may have some standard of comparison it was necessary to know as accurately as possible the amount of chlorides in the soil. For this reason six 10-gm. portions were leached free of chlorides. The filtrate was made to 500 cc. and aliquot parts analyzed. The gravimetric method which is recognized as giving very accurate results under proper conditions gave, as an average of 11 determinations,

4.98 per cent of chlorides calculated as sodium chloride. These determinations agreed within experimental error, hence may be taken as the per cent of chlorides in the soil.

The adsorptive action of filters on sodium chloride. The adsorptive action of Pasteur-Chamberland filters on sodium chloride was determined by filtering solutions of sodium chloride of varying strengths. These solutions were then titrated with standard 0.1 *N* silver nitrate.

The quantity of chlorides in the solution is the same after filtration as before, thus indicating that any adsorptive action which may be exerted by the filter is within experimental error, a conclusion which is in accord with other findings (3, 6).

TABLE 3

Sodium chloride in solutions before and after filtering through Pasteur-Chamberland filters

MOHR METHOD		VOLHARD METHOD	
Before filtration	After filtration	Before filtration	After filtration
mgm.	*mgm.*	*mgm.*	*mgm.*
5.855	5.878	5.867	5.859
2.937	2.937	2.931	2.955
1.962	1.971	1.952	1.962
1.488	1.488	1.471	1.483
1.183	1.181	1.184	1.161

TABLE 4

The chlorides, calculated as sodium chloride, extracted from soils with varying amounts of water

RATIOS	CHLORIDES EXTRACTED FROM SOIL BY VARYING RATIOS OF WATER AND DETERMINED BY			
	Mohr method	Error	Volhard method	Error
	per cent		*per cent*	
1 to 5	5.041	±0.008	4.975	±0.012
1 to 10	5.067	±0.01	4.986	±0.012
1 to 15	5.054	±0.013	4.956	±0.012
1 to 20	5.057	±0.017	4.974	±0.013

Ratio of soil to water. For the extraction of soluble salts from soils the workers have used ratios as narrow as 1 part of soil to 1 of water, and as wide as 1 part of soil to 25 parts of water. In our work the following ratios were used: 1 to 5, 1 to 10, 1 to 15 and 1 to 20, with the results reported in table 4.

The soil yielded to the gravimetric method 4.98 per cent of chlorides, calculated as sodium chloride. Here we find when the ratio of soil to water is 1 to 5 by the Volhard method 4.975 per cent and with the ratio 1 to 20, 4.974 per cent. The Mohr method gave 5.041 and 5.067, respectively, for the same ratios. The per cent of chlorides found in the soil by leaching is

4.984. The average of that found by the Mohr and Volhard methods for the ratios 1 to 5 and 1 to 20 are 5.008 and 5.037 per cent, respectively, which are slightly higher than those yielded on leaching the soil, the difference probably being experimental error. The Volhard method, however, yielded results which are almost identical with those obtained on leaching and determining gravimetrically the chlorides. We may conclude that all the chlorides are extracted by using 5 parts of water to 1 of soil. In our work on the extraction of nitric nitrogen from soils (6), we found this ratio of water to soil sufficient to extract all nitrates present.

Time of extraction. The time necessary for water added to a soil to dissolve the chlorides in a soil may vary greatly with the conditions of the soil when the water is added, as well as the treatment of the soil-water mixture.

In order to find the minimum time necessary for extraction we have extracted 100-gm. portions of soil containing 2 gm. of alum with 500 cc. of water. The soil was mixed with the water in 1000-cc. bottles and shaken in a

TABLE 5

Chlorides extracted from a soil after varying periods

TIME OF SHAKING	GENERAL AVERAGE		THE SOLUTION CLARIFIED WITH 2 GM. ALUM		SOLUTION CLARIFIED BY CHAMBERLAND-PASTEUR FILTERS	
	Sodium chloride	Error	Sodium chloride	Error	Sodium chloride	Error
minutes	*per cent*		*per cent*		*per cent*	
5	4.987	±0.015	5.002	±0.01	4.973	±0.021
10	4.997	±0.007	5.002	±0.005	4.993	±0.009
20	4.981	±0.011	4.969	±0.013	4.994	±0.01
30	4.992	±0.009	4.990	±0.01	4.994	±0.008
45	4.977	±0.012	4.970	±0.011	4.984	±0.012

mechanical shaker for the time indicated, then allowed to stand long enough to settle and then the chlorides determined. The average results are given in table 5.

If we take as the amount of chlorides present, that shown by the gravimetric method on the solutions obtained by leaching the soil free of chlorides, namely, 4.98 per cent, then there is nothing to be gained by shaking the soil longer than five minutes, as may be observed from the results which all agree within experimental error. This is probably sufficient where the soil is finely divided and thoroughly agitated as was the case with this soil.

The influence of various salts on the determination of chlorides in soil by the Volhard and the Mohr methods. In order to ascertain the influence of salts on the determination of chlorides in soil solution, 50 gm. of soil to which was added 5 gm. of the various salts was agitated with 500 cc. of water in a shaking machine for 10 minutes. Part of the solutions were filtered through Pasteur-Chamberland filters and part clarified by the addition of 2 gm. of

alum. The chlorides were determined in aliquot portions by both the Mohr and Volhard methods. The results are given in table 6. It will be noticed that the salts are tabulated in such a way as to show the influence of the anion and the cation. The calcium salts give normal results both by the Mohr and the Volhard methods, that is, the results are entirely comparable with those obtained by the same method in the absence of the salts. The same is also true of the magnesium ion. With the exception of the sodium

TABLE 6

The influence of various salts on the determination of chlorides by the Mohr and Volhard methods

METHOD	SALT USED	ALUM			FILTER		
		Number of analyses	Error	NaCl	NaCl	Error	Number of analyses
				per cent	*per cent*		
Volhard	$CaCO_3$	6	±0.007	5.01	5.02	±0.01	6
	$Ca(NO_3)_2$	6	±0.004	4.99	4.95	±0.02	6
	$CaSO_4$	6	±0.003	5.02	5.01	0.0	6
Average		18	±0.005	5.007	4.993	±0.01	18
Mohr	$CaCO_3$	6	±0.003	5.08	5.12	±0.01	6
	$Ca(NO_3)_2$	6	±0.008	5.08	5.03	±0.02	6
	$CaSO_4$	6	±0.005	5.09	5.09	±0.00	6
Average		18	±0.005	5.08	5.08	±0.01	18
Volhard	$MgSO_4$	4	±0.01	4.99	5.02	±0.002	6
	$Mg(NO_3)_2$	9	±0.008	4.942	4.925	±0.003	6
	$MgCO_3$	5	±0.01	4.946	4.954	±0.01	6
Average		18	±0.009	4.957	4.954	±0.005	18
Mohr	$MgSO_4$	4	±0.00	5.09	5.08	±0.002	6
	$Mg(NO_3)_2$	6	±0.006	5.018	5.004	±0.003	6
	$MgCO_3$	6	±0.01	5.028	5.054	±0.01	6
Average		16	±0.005	5.045	5.046	±0.005	18
Volhard	Na_2CO_3	6	±0.018	4.89	4.95	±0.00	6
	$NaNO_3$	6	±0.01	4.87	4.88	±0.02	6
	$NaHCO_3$	6	±0.008	4.90	4.91	±0.005	6
	Na_2SO_4	6	±0.012	4.92	4.93	±0.01	6
Average		24	±0.012	4.895	4.92	±0.008	24
Mohr	Na_2CO_3	6	±0.00	5.26	5.26	±0.00	6
	$NaNO_3$	6	±0.007	4.99	4.95	±0.02	6
	$NaHCO_3$	6	±0.01	5.02	5.02	±0.025	6
	Na_2SO_4	6	±0.016	5.03	5.02	±0.005	6
Average		24	±0.008	5.075	5.062	±0.014	24

TABLE 6—*Continued*

METHOD	SALT USED	CLARIFIED WITH ALUM			CLARIFIED WITH FILTER		
		Number of analyses	Error	NaCl	NaCl	Error	Number of analyses
				per cent	*per cent*		
Volhard	$CaCO_3$	6	±0.007	5.01	5.02	±0.01	6
	$MgCO_3$	5	±0.01	4.946	4.954	±0.01	6
	Na_2CO_3	6	±0.018	4.89	4.95	±0.00	6
	$NaHCO_3$	6	±0.008	4.90	4.91	±0.005	6
	$FeCO_3$	6	±0.005	4.938	4.967	±0.002	6
Average		29	±0.009	4.938	4.960	±0.005	30
Mohr	$CaCO_3$	6	±0.003	5.08	5.12	±0.01	6
	$MgCO_3$	6	±0.01	5.025	5.054	±0.01	6
	Na_2CO_3	6	±0.00	5.26	5.26	±0.00	6
	$NaHCO_3$	6	±0.01	5.02	5.02	±0.005	6
	$FeCO_3$	6	±0.00	5.042	5.045	±0.002	6
Average		30	±0.005	5.085	5.102	±0.005	30
Volhard	$Ca(NO_3)_2$	6	±0.004	4.99	4.95	±0.02	6
	$Mg(NO_3)_2$	9	±0.008	4.942	4.925	±0.003	6
	$NaNO_3$	6	±0.01	4.87	4.88	±0.02	6
Average		21	±0.007	4.934	4.918	±0.014	18
Mohr	$Ca(NO_3)_2$	6	±0.008	5.08	5.03	±0.02	6
	$Mg(NO_3)_2$	6	±0.006	5.018	5.004	±0.003	6
	$NaNO_3$	6	±0.007	4.99	4.95	±0.02	6
Average		18	±0.007	5.029	4.995	±0.014	18
Volhard	$CaSO_4$	6	±0.003	5.02	5.01	±0.00	6
	$MgSO_4$	4	±0.01	4.99	5.02	±0.002	6
	Na_2SO_4	6	±0.012	4.92	4.93	±0.01	6
Average		16	±0.008	4.977	4.99	±0.004	18
Mohr	$CaSO_4$	6	±0.005	5.09	5.09	±0.00	6
	$MgSO_4$	4	±0.00	5.09	5.08	±0.002	6
	Na_2SO_4	6	±0.016	5.03	5.02	±0.01	6
Average		16	±0.007	5.07	5.063	±0.004	18

carbonate, which gives abnormally high results, the sodium salt decreases the quantity of chlorides recovered.

Of the acid ions the sulfates are without effect whereas the nitrates have a depressing influence. This is in conformity with the reference already cited (5, p. 522) that silver chloride is somewhat soluble in the presence of nitrates of the alkali and alkali earths. The carbonates decreased the quantity of

chloride as measured by the Volhard method. This is to be expected, since the carbonates are converted into nitrates by the added nitric acid. With the exception of sodium carbonate the carbonates do not interfere with the Mohr method. This is undoubtedly due to the low solubility of the carbonates used. With sodium carbonate, however, abnormally high results are obtained. Besides coloring the filtrate brown, thereby obscuring the end-point, sodium carbonate causes a precipitation of silver carbonate, since silver carbonate is less soluble than silver chromate and the amount of silver carbonate precipitated will depend, of course, on the quantity of sodium carbonate present.

The Mohr vs. the Volhard Method. Brautlecht and Langley (2) compared these methods in their adaptability to the determination of chlorides in waters. They found the Volhard gave more satisfactory concordant and accurate results when 0.1 *N* solutions were used but required a longer time. They found 0.01 *N* sulfocyanate and silver nitrate solutions too dilute to give sharp

TABLE 7

Chlorides found in solution by the Mohr and Volhard methods

MOHR METHOD		VOLHARD METHOD	
Chlorides calculated as NaCl	Error	Chlorides calculated as NaCl	Error
per cent		*per cent*	
5.041	±0.011	4.964	±0.011

end-points. Shutt and Charlton (11) reached a similar conclusion when working on a large number of waters containing only small amounts of chlorine. Stuart (12) found that the Volhard method can be used with waters containing small amounts of chlorine only when a very small excess of silver nitrate is used, not exceeding 0.2 cc. The plus or minus error will be about 0.5 parts per million of waters containing 10 parts per million of chlorine.

The vast amount of work done by us makes it possible to compare the results obtained by the Mohr and Volhard methods with those obtained by the gravimetric.

This is done in table 7, in which is given the average for 22 determinations by the two methods. The determinations were made on the same solutions so that the only variable is the method of determining the chlorides.

The Mohr method invariably gives results higher than the Volhard method. This has been rectified by many workers by making a correction which is not necessary in the case of the Volhard method.

If we take the chlorides as determined by leaching and precipitation with silver nitrate (4.98 per cent) as standard we find a very close agreement between this result and that obtained by the Volhard method. Therefore, it would appear from all of our results that the Volhard method is more accurate for the determination of chlorides in soil solution than is the Mohr method.

SUMMARY

Equally satisfactory soil extracts from which the chlorides are to be determined are obtained either by the use of 2 gm. of alum or by the Pasteur-Chamberland filter. The extract obtained by use of the centrifuge gave higher results, due perhaps to the presence of proteins or other colloids in suspension. The quantity of chlorides obtained by the use of 5 parts of water to 1 part of soil was the same as with larger quantities of water.

Nothing is to be gained by agitating the soil and water for more than 5 minutes if the soil is finely divided and the solution vigorously shaken. The Mohr and Volhard methods give low results in the presence of nitrates of the alkali and the alkali earths. The Volhard method gives low results in the presence of carbonates of the alkali and alkali earths. The Mohr method gives high results in the presence of carbonates due to the precipitation of silver carbonate. The Volhard method gives more concordant and, as judged by the gravimetric method, more nearly exact results than the Mohr method. Ethyl alcohol causes the coagulation of silver chloride as well as ether or boiling.

We obtained the best results by the following method: 100 gm. of finely pulverized soil was agitated in a mechanical shaker for 5 minutes and clarified either by the use of 2 gm. of alum or by the Pasteur-Chamberland filter; 20-cc. portions were pipetted into ground-glass-stoppered bottles and an excess of 0.1 *N* silver nitrate added; 10-cc. of 95 per cent ethyl alcohol was added and the mixture shaken until the silver chloride was coagulated and the supernatant solution was clear; 2 cc. of ferric sulfate solution containing 20 gm. of ferric sulfate to 500 cc. of water, and 5 cc .of dilute nitric acid were added; the excess of silver nitrate was then titrated by the use of 0.1 *N* NH_4SCN.

REFERENCES

(1) ALEFELD, E. 1909 Eine Vereinsachung der Gravimetrischen Halogen Silberbestimmung. *In* Ztschr. Analyt. Chem., Bd. 48, p. 79–80.

(2) BRAUTLECHT, C. A., AND LANGLEY, B. N. 1915 The normal chlorine content of surface waters of western Florida and a comparison of volumetric methods of chlorine analysis. *In* Jour. Indus. Engin. Chem., v. 7, p. 357–358.

(3) BRIGGS, LYMAN J., AND BRIGGS, MACY H. 1902 Capillary studies and filtration of clay from soil solutions. U. S. Dept. Agr. Bur. Soils Bul. 19, p. 34.

(4) DRECHSEL, E. 1877 Zur Volhard'schen Silberbestimmungsmethode. *In* Ztschr. Analyt. Chem., Bd. 16, p. 351–353.

(5) FRESENIUS, K. R. 1903 Quantitative Chemical Analysis, v. 1. H. W. Wiley and Sons, New York.

(6) GREAVES, J. E., AND HIRST, C. T. 1917 Some factors influencing the quantitative determination of nitric nitrogen in the soil. *In* Soil Sci., v. 4, no. 3, p. 179–205.

(7) KOLTHOFF, I. M. 1907 The titration by Mohr's method and its application to potable waters. *In* Pharm. Weekbl., Bd. 54, p. 612–618.

(8) LUNGE, GEORGE 1908 Technical Methods of Chemical Analysis, v. 1, pt. 1, p. 123. D. Van Nostrand Co., New York.

(9) RASANOFF, M. A., AND HILL, ANTHON E. 1907 A necessary modification of Volhard's method for the determination of chlorides. *In* Jour. Amer. Chem. Soc., v. 29, p. 269–275.

(10) ROTHMUND, V., AND BURGSTELLER, A. 1909 The accuracy of the Volhard method for the determination of chlorine. *In* Ztschr. Anorgan. Chem.,Bd. 63, No. 4, p. 330–336.

(11) SHUTT, F. T., AND CHARLTON, H. W. 1906 The Volhard method for the determination of chlorine in potable waters. *In* Trans. Roy. Soc. Canada, ser. 2, v. 11, sec. 111, p. 67–71.

(12) STUART, A. T. 1911 Volhard's method for the estimation of chlorine in potable waters. *In* Jour. Amer. Chem. Soc., v. 33, no. 8, p. 1344–1349.

(13) SUTTON, FRANCIS 1911 A Systematic Handbook of Volumetric Analysis, 6th Edition, p. 124. P. Blakiston, Son and Co., Philadelphia.

(14) TILLMANS, J., AND HEUBLEIN, O. 1913 The determination of chlorine in natural waters. *In* Chem. Ztg., Bd. 37, p. 901–903.

(15) TREADWELL, F. P. 1904 Analytical Chemistry; v. 2, Quantitative Analysis (Translation by Hall, W. T.), p. 707. H. W. Wiley and Sons, New York.

(16) VAN ECK, P. N. 1916 Silver chromate. *In* Pharm. Weekbl., Bd. 53, p. 1554–1558.

(17) WINKLER, L. 1894 The determination of chlorine ion in natural waters. *In* Ztschr. Analyt. Chem., Bd. 33, p. 357.

(18) YODER, LESTER 1919 Adaptation of the Mohr volumetric method to general determinations of chlorine. *In* Jour. Indus. Engin. Chem., v. 11, p. 755.

THE FORMATION OF NITRATES IN A SOIL FOLLOWING THE GROWTH OF RED CLOVER AND OF TIMOTHY

T. L. LYON, J. A. BIZZELL AND B. D. WILSON

Cornell University

Received for publication January 5, 1919

It has usually been observed that the growth of clover or alfalfa on a piece of land improves it in so far as crop production is concerned. This is true even when the hay crops are all removed and only the stubble and roots incorporated in the soil. Because these plants are nitrogen fixers, we are naturally inclined to associate the improved productiveness to an increase in the supply of total or available nitrogen.

There still remain several questions regarding the reason for soil improvement by legumes which have not been definitely settled. There appears to be some doubt as to whether the total nitrogen content of a soil is always greater after a legume has been raised on it than before, even when its growth has made the soil capable of producing larger crops. If there has been no increase in the quantity of nitrogen present, has there been a change in the readiness with which organic nitrogen is converted into nitrates, and if so, is that due to the quality of the organic nitrogen in the plant residues or does it result from some other influence of the legume on the production of nitrates?

It is not our purpose to discuss in this paper the effect of legumes on the nitrogen balance in soil on which they have been grown. It is, rather, the effect of these crops on the quantity of nitrogen in the form of nitrates after the legumes have been cut for hay and the stubble or roots plowed under that is here to be considered. Experiments reported by a number of investigators cover two aspects of the subject. One of these is the capacity of a soil to form nitrates from the nitrogen remaining in the soil after the growth of the legumes; the other is the power of the soil to form nitrates from organic nitrogenous matter or ammonium salts added to the soil when it is brought into the laboratory and incubated.

In the following brief review of some of the investigations dealing with this subject no consideration is given to records of the determination of nitrates in soil on which crops are growing unless samples of soil are removed and nitrates allowed to develop during incubation of the soil without the presence of crop plants. The presence of growing crops introduces a factor which complicates any attempt to find the effect of the residues of the crop on nitrate formation. To simplify the discussion this limitation will be observed, and the effect of fallow or condition other than crop growth will be omitted.

Warington (10) analyzed the drainage water passing through a soil from which a wheat crop had been removed, one-half of the area having grown a crop of clover immediately before the wheat was planted and the other half having been in barley. He found that the drainage from the clover soil contained more nitrate nitrogen than did the barley soil.

King and Whitson (6) conducted experiments with soil in cylinders in the greenhouse which they planted respectively to corn, clover and oats. After these crops were removed the cylinders were allowed to stand for 93 days under the same conditions of moisture and temperature. During this period one set of cylinders representing the three crops was left uncultivated, one set was cultivated once a week, and one set was cultivated once in two weeks. At the end of the period the quantity of nitrates in the cultivated soil was in the ratio of 31:47:28 for corn, clover and oats, respectively, but in the uncultivated soil there was practically no difference in the quantity of nitrates in the soil previously planted to these crops.

As the method of treating the sample of soil previous to the determination of nitrates may possibly be a factor in the results, that operation will be described in each review if it has been found in the text. In this experiment the determination of nitrates appears to have been made without previously drying the soil.

Brown (1), in experimenting with soil from a series of field plats which had been cropped in various ways for a number of years, found that incubation with ammonium sulfate and also with dried blood gave, on the whole, a slightly lower production of nitrates from the soil on which clover had been grown continuously than from soil on which corn had been grown continuously, but where clover had been used in a rotation nitrate production was much higher than in the soils just mentioned and also than in soil on which the same rotation was used without clover.

In these experiments and also in those by Brown to be reviewed later the soil before incubation was air-dried and screened.

Greaves (3) determined the nitrifying power of soil from a number of fields whose previous treatments had been very diverse but which at the time of sampling were planted to alfalfa and wheat. He reports that the nitrifying power of soil on which alfalfa was growing was much less than that of soil on which wheat was growing.

In a continuation of the experiment by Brown (2) previously reviewed he found that both dried blood and ammonium sulfate produced more nitrate nitrogen when incubated with soil cropped to a rotation of corn, oats and clover than with a soil cropped only to corn and oats, or soil planted continuously to corn.

McBeth and Smith (8) experimented with soils from plats irrigated with different quantities of water and also unirrigated. Each set of plants so treated produced crops of alfalfa, potatoes, corn and oats. The experiment extended through the years 1909 to 1912, inclusive. The nitrifying power

of the soil with dried blood and also with ammonium sulfate was determined. Soil samples were air-dried to 3 per cent moisture before the first determination of nitrates was made and before the sample was incubated. While the results of the nitrification tests were not uniform for the different years and the different applications of irrigation water they showed, in the main, a greater nitrifying power for the alfalfa soil than for the potato, corn and oat soil, which three crops appear not to have differed much in their effect on the nitrifying power of the soil.

Velbel (9) found that nitrate formation was increased by the cultivation of leguminous plants.

Kellerman and Wright (5) planted peas, vetch, barley, rye and Kafir corn in wire baskets, using a series of these crops in each of two types of soil. After growing for 80 days the plants and roots were removed and nitrification tests were made with the addition of peptone to one set of soil samples and of ammonium sulfate to another set. There appeared to be no consistent difference between the soil planted to the legumes and that planted to the non-legumes in respect to the quantity of nitrates formed during incubation.

Greaves, Stewart and Hirst (4), experimenting apparently with the same plats at Greenville, Utah, as those used in the experiments by McBeth and Smith, which have already been reviewed, and following much the same methods of preparing soil for incubation, found that the nitrifying power of soil with dried blood was greatest when taken from where potatoes were growing and less for alfalfa, oats and corn in the order named.

Lyon and Bizzell (7) found more nitrate nitrogen in soil on which alfalfa had previously grown for 6 years and which was then kept bare of vegetation than in soil on which timothy had grown for the same period and which was also free of vegetation. They also found that the alfalfa soil produced more nitrates when incubated both with and without dried blood.

There is sufficient discrepancy in the results of the experiments reported to leave some doubt whether the legumes used in the experiments increased the nitrifying power of the soil for dried blood as compared with the influence of the non-legumes. Experiments in which the crop residues alone were concerned leave less room for doubt, the conclusions of the three investigations on that subject being unanimous in favor of the legume. In the following experiment an attempt has been made to deal with the question in a somewhat different manner than has been done in the investigations cited.

PLAN OF THE EXPERIMENT

The experiment involved the leaching of soil on which different crops were growing. To accomplish the leaching while the crops were on the soil and to obviate the necessity of removing and consequently of aerating it, the soil was placed in cylindrical cans with funnel-shaped bottoms from which the drainage water was conducted by pipes connected with large bottles in which

the leachings were collected. The cylinders were 12 inches in diameter and 2 feet deep. Ten inches at the bottom were filled with a weighed quantity of clay subsoil and the funnel-shaped bottom was filled with sand and gravel.

It was intended that the soil should be very rich in readily nitrifiable nitrogen, for which purpose dried blood was added to the soil at the rate of 45 gm. per can. Acid phosphate was added at the rate of 15 gm., muriate of potash at the rate of 8 gm., and ground limestone at the rate of 90 gm. per can. The same weight of soil was placed in each can.

The soil used in the experiment was a sand of moderate fertility and good drainage. The commercial fertilizer used in such liberal quantities made it productive and the lime permitted an abundant growth of clover. No difficulty was experienced in leaching the soil and the drainage water was clear.

Twelve cylinders were employed, six of which were planted to timothy and six to red clover. The soil of all cans was inoculated with a pure culture of *Bacillus radicicola* from the clover plant. Each can also received 1000 cc. of an infusion made by stirring 1 pound of garden soil with 3 liters of tap water. This was to insure an active nitrifying flora. The crops were planted on September 15 and on September 29 there was a good stand.

During the time the timothy and clover were growing the soil was leached from time to time, and as water was dripping from the outlets much of the time the soil in all cans contained about the same quantity of moisture. All the water applied had been distilled and was free from nitrates. The growth of the plants was sufficient to permit leaching on November 27, and from that time until the crops were harvested the soil of each can was leached about once a month.

It was intended to estimate the nitrogen contained in the drainage water and all of this proved to be in the form of nitrates which were determined by the phenol disulfonic acid method. There were seven leachings during the time the timothy and clover were on the soil.

NITROGEN IN CROPS

The crops were harvested on May 13. Both crops had made a good growth, the clover relatively better than the timothy, but both were healthy and vigorous. At the time of harvest the clover was in blossom and the timothy had not yet reached that stage. The crop from each can was dried, weighed and its nitrogen content determined. The yields of dry matter and of nitrogen in these crops are shown in table 1.

The total quantity of nitrogen removed from the soil in the cans on which timothy was grown was small as compared with the quantity added in the form of dried blood. This material contained 10.5 per cent of nitrogen and, consequently, at the rate of 45 gm. of dried blood per can the total amount of nitrogen thus added to the six timothy cans would be 28.35 gm. The crop removed a total of 4.0962 gm. from the six cans, thus leaving a large

quantity of easily nitrifiable nitrogen remaining in the soil. It is impossible to say how much nitrogen the clover took from the soil.

TABLE 1

Weights of dry matter and nitrogen in timothy and red clover grown in cans 9–20

Can Number	TIMOTHY		Can Number	RED CLOVER	
	Dry matter	Nitrogen		Dry matter	Nitrogen
	gm.	*gm.*		*gm.*	*gm.*
9	46.95	0.5978	15	179.86	5.3669
10	60.25	0.7497	16	169.99	5.0688
11	66.65	0.7866	17	168.75	4.9104
12	68.03	0.7840	18	165.27	4.8894
13	49.19	0.5712	19	179.83	5.3865
14	49.09	0.6069	20	170.68	5.1015
Total.....	340.16	4.0962		1034.38	30.7235

TABLE 2

Nitrogen in leachings during growth of timothy and red clover

CAN NUMBER	CROP	NITROGEN IN DRAINAGE WATER BETWEEN DATES INDICATED							TOTAL NITROGEN	TOTAL VOLUME OF DRAINAGE WATER
		Sept. 29 to Nov. 27	Nov. 27 to Dec. 11	Dec. 11 to Jan. 14	Jan. 14 to Feb. 11	Feb. 11 to Mar. 11	Mar. 11 to April 11	April 11 to May 11		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*liters*
9	Timothy	0.23	0.12	0.14	0.004	0.0005	0.0005	trace	0.4950	28.043
10	Timothy	0.23	0.10	0.08	0.003	trace	trace	trace	0.4130	27.342
11	Timothy	0.22	0.07	0.02	trace	trace	trace	trace	0.3100	23.188
12	Timothy	0.24	0.07	0.03	trace	trace	trace	trace	0.3400	26.431
13	Timothy	0.26	0.13	0.08	0.004	trace	trace	trace	0.4704	28.882
14	Timothy	0.17	0.07	0.06	0.004	trace	trace	trace	0.3004	29.583
Total		1.35	0.56	0.41	0.0078	0.0005	0.0005	trace	2.3288	163.469
15	Red clover	0.17	0.06	0.09	0.008	0.0012	0.0009	trace	0.3301	27.391
16	Red clover	0.14	0.06	0.07	0.019	0.0023	0.0014	0.0016	0.2943	25.720
17	Red clover	0.17	0.06	0.07	0.018	0.0027	0.0011	0.0007	0.3225	28.923
18	Red clover	0.16	0.09	0.08	0.016	0.0025	0.0011	0.0009	0.3505	30.100
19	Red clover	0.13	0.04	0.07	0.016	0.0009	0.0005	0.0007	0.2581	22.535
20	Red clover	0.16	0.04	0.01	0.003	0.0011	0.0005	0.0003	0.2149	18.884
Total		0.93	0.35	0.39	0.080	0.0107	0.0055	0.0042	1.7704	153.553

NITROGEN IN DRAINAGE WATER DURING GROWTH OF TIMOTHY AND RED CLOVER

The soil in the cans was leached with distilled water at intervals during the time the timothy and clover were growing. A record was kept of the amount of water that passed through the soil and of the nitrates (NO_3) in parts per million contained in the percolate. Table 2 shows the quantity of nitrogen

removed in the drainage water from each can at each leaching, also the total volume of water percolating through the soil during the time the timothy and clover crops were growing.

It may be seen from the figures in the table given above that most of the nitrogen leached out of the timothy soil was in the first two leachings and that the last three leachings contained practically none. Red clover, on the other hand, continued to give appreciable quantities in all the leachings. Apparently nitrification continued more actively in the clover soil than in the timothy soil while the crops were making their later growth, or else the clover made less use of the nitrate nitrogen of the soil than did the timothy. The quantity of nitrate nitrogen leached from the clover soil after growth was well started was so small as to indicate that either nitrification had practically stopped or that the clover was absorbing nitrate nitrogen.

NITROGEN IN DRAINAGE WATER AFTER THE SOIL HAD REMAINED FALLOW FOR A MONTH

The crops having been harvested, the soil was stirred up to a depth of about 2 inches and the stubble and coarse roots near the surface were removed, but the remainder of the roots were left in the soil. The soil was allowed to stand

TABLE 3

Nitrogen in drainage water from fallow soil

CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER	CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER
		liters	*gm.*			*liters*	*gm.*
9	Timothy	6.958	0.0156	15	Red clover	7.270	0.1215
10	Timothy	6.158	0.0099	16	Red clover	7.605	0.0927
11	Timothy	7.582	0.0121	17	Red clover	7.250	0.0884
12	Timothy	7.850	0.0120	18	Red clover	8.205	0.1297
13	Timothy	7.060	0.0172	19	Red clover	6.452	0.0933
14	Timothy	7.228	0.0359	20	Red clover	7.445	0.1446
Total		42.836	0.1027	Total		44.227	0.6702

in this condition from May 13 to June 15, water being added from time to time, but no vegetation being allowed to develop. It was then leached and nitrates were determined in the leachings. The volume of drainage water from each can and the nitrogen it contained are stated in table 3.

When the soil was allowed to lie fallow the formation of nitrates was much more active in the soil previously in red clover than in that which had raised timothy. The figures given above indicate a production of about six times as much nitric nitrogen in the clover soil. The timothy soil had a large quantity of easily nitrifiable nitrogen still present in the form of dried blood, as only a small proportion of this had been used by the previous crop or had been

leached out. The greater quantity of nitrates leached from the clover soil during this period is evidently not to be accounted for by the assumption that the clover crop did not absorb nitrates. The fact that the soil had been leached several times while the clover was growing and that the quantity so obtained was small, would invalidate this assumption. There was, without doubt, a much more active formation of nitrates following the growth of clover than of timothy.

THE PRODUCTION OF CROPS ON SOILS PREVIOUSLY PLANTED TO TIMOTHY AND TO RED CLOVER

On June 22 four of the cans were planted to oats and four to maize, the remaining four being allowed to continue in fallow and being left free of vegetation during the entire time the other crops were on the soil. Both the oats and maize plants were beginning to appear above ground on June 27. The

TABLE 4

Volume of water added to each can between June 22 and October 21

CAN NUMBER	PREVIOUS CROP	PRESENT CROP	WATER ADDED	CAN NUMBER	PREVIOUS CROP	PRESENT CROP	WATER ADDED
			liters				*liters*
9	Timothy	None	30	15	Red clover	None	28
10	Timothy	Oats	45	16	Red clover	Oats	54
11	Timothy	Maize	40	17	Red clover	Maize	46
12	Timothy	None	29	18	Red clover	None	27
13	Timothy	Oats	47	19	Red clover	Oats	53
14	Timothy	Maize	39	20	Red clover	Maize	43

oats were thinned to 18 plants per can and the maize to 3 plants. Early in July the cans were taken out of the greenhouse and remained outside until the latter part of September, during which time they received the rainfall. The quantity of distilled water added to each can in addition to the rainfall is shown in table 4.

It will be seen at once that the oats and maize on the soil previously in red clover required more water than did the same crops on the timothy soil. This was reflected in the yields of crops, which was greater on the clover soil. The fact that the oats required more water than the maize is probably to be accounted for by the small growth of the latter.[1] The fallow soils all received nearly the same quantities of water.

Throughout the entire period of growth the plants on the clover soil were more vigorous. On July 15 maize on timothy soil had 5 leaves and that on

[1] The maize was a very small and early maturing variety, which probably accounts for the small production of dry matter and nitrogen in the crop. It would doubtless have been better to have used a somewhat larger variety.

clover soil 7 leaves, and the latter was a deeper green and taller. On the same date the oats on the clover soil had tillered nearly twice as much as on the timothy soil and was likewise a better color. Oats, however, did not show as much benefit from the clover at an early stage as did maize.

Maize on clover soil began to show some tassels on August 15, that on timothy soil on August 20. Oats on clover soil began to head on August 17 and that on timothy soil on August 21.

On September 23 the maize had ripened, but the oats were only partly yellow. The rather unusual occurrence of maize ripening before oats was probably brought about by the late planting of the oats, which necessitated its early growth being made during warm weather and to the naturally early maturing character of the maize.

TABLE 5

Nitrogen in oats and maize following timothy and red clover

PREVIOUS CROP	CAN NUMBER	NITROGEN IN OATS AND MAIZE
		gm.
Timothy	10, 11, 13, 14	1.3346
Red clover	16, 17, 19, 20	2.3951

TABLE 6

Volume and analysis of drainage water collected between June 22 and November 14

CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER
		liters	*gm.*
9, 10, 11, 12, 13, 14	Timothy	89.018	1.4353
15, 16, 17, 18, 19, 20	Red clover	86.793	3.2460

The crops were left on the soil until October 21, at which time there was no question that the maize was fully ripe, but the oats were still partly green. The crops were harvested on this date by cutting off the stems at the surface of the soil, after which the grain and straw or stalks were separated. They were then dried, ground and subjected to analysis, the results of which are shown in table 5.

The much larger absorption of nitrogen by the crops on the clover soil is at once apparent. The excessive application of dried blood to the previous crop might well be expected to have had a residual effect on the crops grown on the timothy soil but this was slight as compared with the influence which red clover exerted on the nitrogen content of the crops which followed that legume.

After the crops were removed the soil of each can was subjected to repeated leachings in order to remove the nitrates as completely as practicable, and on

the figures of the last leaching the quantity of nitrates remaining in the water in the soil was calculated. The results of the leachings during the time when the oats and maize were growing and after their removal, together with the nitrates remaining in the soil water, are shown in table 6.

The total quantity of nitrate nitrogen in the drainage water following clover was more than twice as great as in the water following timothy.

REMOVAL OF NITROGEN FROM SOIL FOLLOWING THE GROWTH OF TIMOTHY AND RED CLOVER

It still remains to summarize the total removal of nitrogen from the soil following the growth of timothy and clover. The media in which this nitrogen is contained are (*a*) the drainage water from the soil which lay fallow for a month following the growth of timothy and clover, (*b*) the oat and maize crops raised after the fallow, and (*c*) the drainage water from the soil on which

TABLE 7

Nitrogen contained in crops and drainage water following timothy and red clover

	CAN NUMBERS	
	9, 10, 11 12 13, 14	15 16, 17 18, 19, 20
Previous crop	Timothy	Red clover
Nitrogen in drainage from fallow soil (gm.)	0.1027	0.6702
Nitrogen in crops of oats and maize (gm.)	1.3346	2.3951
Nitrogen in drainage from oats and maize and bare soil (gm.)	1.4353	3.2460
Total quantity of nitrogen removed (gm.)	2.8726	6.3113

the oats and maize were growing and from the bare soil during the same time. These removals of nitrogen are itemized and totaled in table 7.

Comparing the total quantities of nitrogen removed from the two soils it will be seen that there was a little more than twice as much from the clover soil as from the timothy soil. The quantity in the drainage from the fallow soil during the period from May 13 to June 15 was six times as great from clover soil as from the timothy soil, while the drainage from the oat, maize and bare soil during the period from June 15 to November 14 contained only a little more than twice as much nitrogen from the clover soil as from the timothy. The effect of the clover in increasing nitrate production apparently began to subside after a few weeks. In the drainage water analyzed on October 21 the nitrogen from the clover soil was only about one and one-half times as much as from the timothy soil. Table 8 shows the quantities of nitrogen removed from the soils kept bare of vegetation from the time the timothy and clover were removed in May until the middle of November.

It is very apparent from this table that the clover soil was losing its nitrate-producing power much more rapidly than was the timothy soil. Nitrate production was nine or ten times as rapid in the clover soil immediately after taking off the clover crop, while six months later it was only about one-fourth greater. The effect of clover in increasing nitrate production is not of long duration under the conditions of this experiment and if it is as short-lived in field soils the practice of not plowing up a clover sod until the farmer is ready to replant the land would seem to be advisable. If, however, the conditions had been less favorable for nitrification, the effect of the clover might have been more enduring. In this case there is some question whether the effect of clover on nitrate formation is greater after the first succeeding grain crop than is the effect of the timothy. It should be noted, however, that the clover

TABLE 8

Relative nitrate-producing capacity of uncropped soil previously growing timothy and red clover

PERIOD BETWEEN LEACHINGS	ACTUAL PRODUCTION		RELATIVE PRODUCTION	
	Timothy, cans 9, 12	Clover, cans 15, 18	Timothy, cans 9, 12	Clover, cans 15, 18
	gm.	*gm.*	*gm.*	*gm.*
May 13–June 15	0.0276	0.2512	100	910
June 15–July 15	0.0199	0.1998	100	1004
July 15–July 26	0.0670	0.1720	100	257
July 26–August 14	0.0883	0.3340	100	387
August 14—September 7	0.3113	0.6800	100	218
September 7–September 23	0.2183	0.4452	100	204
September 23–October 21	0.1363	0.1954	100	143
October 21–November 4	0.1137	0.1769	100	155
November 4–November 14	0.1861	0.2284	100	123
Remaining in soil water	0.0419	0.0405		
	1.2104	2.7234		

was on the soil for only a short time and the soil was spaded up immediately after removing the first crop of hay. It should also be noted that the grain crops in the clover soil were twice as large as those in the timothy soil, which is an unusually marked effect of the clover and is probably to be explained by the fact that available nitrogen was the limiting factor in the growth of the grain crops, all the other plant nutrients having been supplied in excess.

If the more abundant formation of nitrates in the clover was due to the more easily nitrifiable character of the nitrogenous organic residue of that crop it would seem probable that this material constitutes only a minor part of the total nitrogenous organic matter in the clover roots. At least this is the case if we assume that one-third of the nitrogen of the clover crop remains in the soil. Calculated on this basis about 15 gm. of nitrogen would have remained in the six cans in which clover was grown. There were 3.4387 gm. of nitrogen in the crops and drainage water of the clover soil in excess of that

in the timothy soil. Something over one-fifth of the total nitrogenous residue of the clover crop had therefore nitrified by the time that the rate of nitrification in the clover soil had declined to the rate of the timothy soil. The remainder of the residue from the clover would not appear to be much more easily nitrified than the nitrogenous residue from the timothy.

The experiment brings out certain data which may be significant. In the first place it has shown that more abundant formation of nitrates took place in clover soil than in timothy soil even when both soils had received a heavy application of dried blood containing several times as much easily nitrifiable nitrogen as was contained in the crop of timothy removed. It has also demonstrated that under the same conditions the growth of oats and maize varied directly with the quantity of nitrates when these crops were produced under conditions in which nitrogen was presumably the limiting factor. It has, furthermore, shown that only a minor part of the total nitrogen probably remaining in the roots of the clover crop was nitrified before the rate of nitrate production in the clover soil fell to that of the timothy soil.

SUMMARY

Twelve cylinders capable of being leached were filled with a soil of medium fertility and good drainage qualities. The soil was abundantly limed and fertilized with acid phosphate, muriate of potash and dried blood. Six cylinders were planted to timothy and six to red clover. The soil of all cans was inoculated with *B. radicicola* from clover nodules.

During the time the timothy and clover were growing the soil was leached with distilled water from time to time. Nitrogen was determined in the drainage water and in the crops of timothy and clover. After these crops were removed the soil was allowed to remain in fallow for a month, leached and nitrogen determined in the drainage. Of the cylinders on which timothy had been grown two were planted to oats, two to maize and two kept free of vegetation. The clover cylinders were treated in the same way. All were leached from time to time and nitrogen determined in the drainage water and also in the crops.

There was little difference in the quantities of nitrogen leached from the timothy soil and clover soil during the time those two crops were growing on them. There was about six times as much nitrogen leached from the clover soil during the month that both soils stood fallow after the timothy and clover crops had been removed. There was only about twice as much nitrogen leached from the fallow clover soil as from the timothy soil during the next five months. At the end of this period the rate of nitrate production in the clover soil was little greater than in the timothy soil. The crops of oats and maize following clover were larger and contained more nitrogen than did those following timothy.

The experiment taken as a whole shows that under the same conditions of soil and treatment clover caused a greater production of available nitrogen than did timothy. This effect is shown in the nitrate content of the drainage water and the total nitrogen content of the oats and maize. Whether the clover stimulated the nitrification process or whether it contributed easily nitrifiable material is not apparent from the data. If the greater production of nitrates in the clover soil was due to the decomposition of the residue of that crop it appears that a portion of this residue is more easily nitrifiable than dried blood and that it constitutes only a small part of the entire residue of the clover crop.

REFERENCES

(1) Brown, P. E. 1912 Bacteriological studies of field soils. II. The effects of continuous cropping and various rotations. Iowa Agr. Exp. Sta. Res. Bul. 6, p. 233–239.

(2) Brown, P. E. 1915 Bacterial activities and crop production. Iowa Agr. Exp. Sta. Res. Bul. 25, p. 367–369.

(3) Greaves, J. E. 1914 A study of the bacterial activities of virgin and cultivated soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, p. 444–459.

(4) Greaves, J. E., Stewart, R., and Hirst, C. T. 1917 Influence of crop, season, and water on the bacterial activities of the soil. *In* Jour. Agr. Res., v. 9, p. 293–341.

(5) Kellerman, K., and Wright, R. C. 1914 Mutual influence of certain crops in relation to nitrogen. *In* Jour. Amer. Soc. Agron., v. 6, p. 204–210.

(6) King, F. H., and Whitson, A. R. 1900 Soluble salts of cultivated soils. Wis. Agr. Exp. Sta. 17th Ann. Rpt. (1899), p. 204–226.

(7) Lyon, T. L., and Bizzell, J. A. 1913 The influence of alfalfa and of timothy on the production of nitrates in soil. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 37, p. 161–167.

(8) McBeth, I. G., and Smith, N. R. 1914 The influence of irrigation and crop production on soil nitrification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, p. 24–51.

(9) Velbel, B. 1912 The course of nitrification in fallow soil. *In* Khutorianin, 1912, no. 10, p. 12–18, 20–24, 26–28; *abs. in* Exp. Sta. Rec., v. 31, p. 722.

(10) Warington, R. 1892 Six lectures on the investigations at Rothamsted Experimental Station. U. S. Dept. Agr. Off. Exp. Sta. Bul. 8, p. 79–84.

SAMPLING SOIL PLOTS

FIRMAN E. BEAR AND GEORGE M. McCLURE

Department of Agricultural Chemistry and Soils, Ohio State University

Received for publication January 2, 1920

Methods of sampling the soil of fertility plots for investigation as to its chemical content are by no means standardized. One is often led to question the conclusions drawn by investigators from their analytical results when the method of choosing the samples was not indicated or, when given, showed a lack of appreciation of the necessity of accuracy in their selection.

The Official Methods of the Association of Agricultural Chemists (4) leave much to be desired in specific suggestions concerning this matter. The directions specify the taking of a "composite sample" with no statement as to the number or location of the individual parts of the composite.

Wiley (10) reviews the methods employed by the more prominent agricultural experiment stations of Europe. In most cases holes are dug with a spade and the sample is secured by making a composite from slices taken from the sides of these holes. The location or number of these holes is not specified.

Warington (8) points out the difficulties involved in sampling the same plot after some years due to the fact that the quantity of organic matter may be changed and the same volume of soil may not represent the same weight of inorganic matter as was contained in the original sample. He also notes that samples should be chosen for comparison when they are in the same condition, i.e., following the same crop and treatment as to cultivation, and suggests securing the sample after a crop has been harvested and before plowing. Warington gives the method adopted by the Rothamsted station and followed since 1865. In this method a square frame of sheet-iron covering an area of 144 square inches is driven into the soil to a depth of 9 inches and the soil contained within this area constitutes the sample.

Many of the methods given are intended for soil survey purposes. The method employed by the United States Bureau of Soils is given by Whitney (9). Hopkins (2) adopted the very definite plan of taking samples to uniform depths but gives no specifications as to distribution of borings in selecting a composite. It is probably not the intention of any of those engaged in soil survey to return to the identical areas from which the samples were chosen, to collect a second sample at some future date for purposes of comparison. Nevertheless, there are on record numerous cases in which conclusions have been drawn from samples chosen at different dates from approximately the same

area, in which no more adequate precautions were taken than those indicated above for insuring that they actually represented the areas in question.

Unless it is possible to sample a given area of soil as many times as may be desired on the same day with the result that the samples are practically identical in composition, there is no reason to believe that conclusions drawn from a comparison of analyses of samples chosen from the same areas at periods several years apart will be trustworthy. This is particularly true if the work is done by different men whose ideas as to how to sample a plot of ground differ.

Leather (3) chose duplicate samples made up of a composite, of 12 borings each, to a depth of 9 inches from areas on three Indian experiment stations. Determinations of the content of nitrogen, phosphoric acid and potash were made. The laboratory error was least on the nitrogen. The duplicate samples varied from 0.001 to 0.015 per cent of this element, with an average variation of 0.006 per cent.

Thiel (7) selected an area of 200 square meters of uniform soil in which, after the soil had been spaded to plow depth, 10 stakes were driven as locations for sampling. A spadeful of soil was selected from near each stake. The composite sample was then passed through a 2-mm. sieve, thoroughly mixed and dried. These operations were repeated twice each month for a year. Five determinations were made on each composite with fairly concordant results. The nitrogen content of the bimonthly composites, calculated to a depth of 50 cm., varied from 9,090 to 10,269 kgm. per hektare.

Pfeiffer and Blanck (5) chose five samples of soil from each of 6 plots located on an area of 9 square meters of land. Four holes were dug on each plot to a depth of 25 cm., and a slice was taken from the side of each hole for the composite. Determinations were made of the nitrogen in the composites. In no case did the nitrogen in any composite vary from the mean of the 5 composites of that plot by more than 0.0063 per cent.

Robinson and Lloyd (6) determined the experimental error in sampling soil in two fields, one of which was quite uniform in texture and the other was not. We will confine the discussion to the uniform field. No records are given as to depth of sampling or type of auger used. Fifteen borings were made at equi-distant points from an area of $\frac{3}{4}$ acre. Each boring was analyzed separately for its content of phosphoric acid. Assuming that the weight of each boring was the same, one can make certain calculations as to what the percentage of phosphoric acid would have been with any combination of samples of a composite. The results of these calculations show that the composite made up of the entire 15 borings would have contained 0.219 per cent of phosphoric acid. By selecting other composites of 5, 6, 7, 9 and 11 borings chosen according to regular schemes over the area, the percentage of phosphoric acid reported might have varied from 0.212 to 0.229. The analytic error is shown in this investigation, however, to be greater than the sampling error.

Bear and Salter (1) chose 6 composites, each consisting of 38 borings, to a depth of $6\frac{2}{3}$ inches, from a plot of $\frac{1}{10}$ acre. These borings were distributed at regular intervals over the plot. The soil was passed through a 2-mm. sieve and then pulverized to pass a 100-mesh sieve. Each composite was analyzed for total carbon, nitrogen and phosphorus. Five of the six composites were quite uniform in their content of these three elements. The greater variation in the total carbon was 0.07 per cent, in total nitrogen 0.009 per cent, and in total phosphorus 0.002 per cent. In the sixth composite the content of these three elements was higher than the lowest of the five other composites by 0.15 per cent of carbon, 0.018 per cent of nitrogen and 0.004 per cent of phosphorus. The greatest variation in duplicates of any composite was 0.02 per cent of carbon, 0.001 per cent of nitrogen and 0.001 per cent of phosphorus.

OBJECT OF THIS EXPERIMENT

It seems desirable to have some very definite plan of selecting soil samples from fertility plots if analytical comparisons are to be made from time to time of the effect of the fertilizer or other treatments the plots receive. It is logical to decide on the method to be employed before field work is begun in order that samples of the original soil may be selected and set aside under appropriate conditions for comparison with samples to be chosen subsequently from the same plot.

The first question which it was thought best to investigate was that of determining how to choose a composite which would accurately represent a plot for the particular day on which it was chosen. No consideration therefore, was given to the question of the relationship of this sample to one which might be chosen from the same area 10 to 20 years hence.

PLAN OF THIS EXPERIMENT

Two plots of ground each $\frac{1}{20}$ acre in size (21 ft. by 103.7 ft.) were set aside for this test. Plot I was as uniform as could be secured on the experimental field. Plot II was chosen for its lack of uniformity. Stakes were driven at various points in the plots and strings were stretched from stake to stake. At points of intersection samples were chosen. This located the samples at the 45 points indicated in figure 1. The samples were chosen with a $1\frac{1}{4}$-inch auger. The surface samples represented a depth of $6\frac{2}{3}$ inches and the subsurface from $6\frac{2}{3}$ to $13\frac{1}{3}$ inches. Care was taken to have the soil at a moisture content which would permit of removing the auger from the hole without losing any of the core. In this latitude the best time for sampling is usually in the month of November. Surface material, leaves and grass were carefully removed before sampling was begun. Both depths were secured by sinking the auger to the proper depth before removing it with the core. If any surface material chanced to fall into the hole before the selection of the second depth,

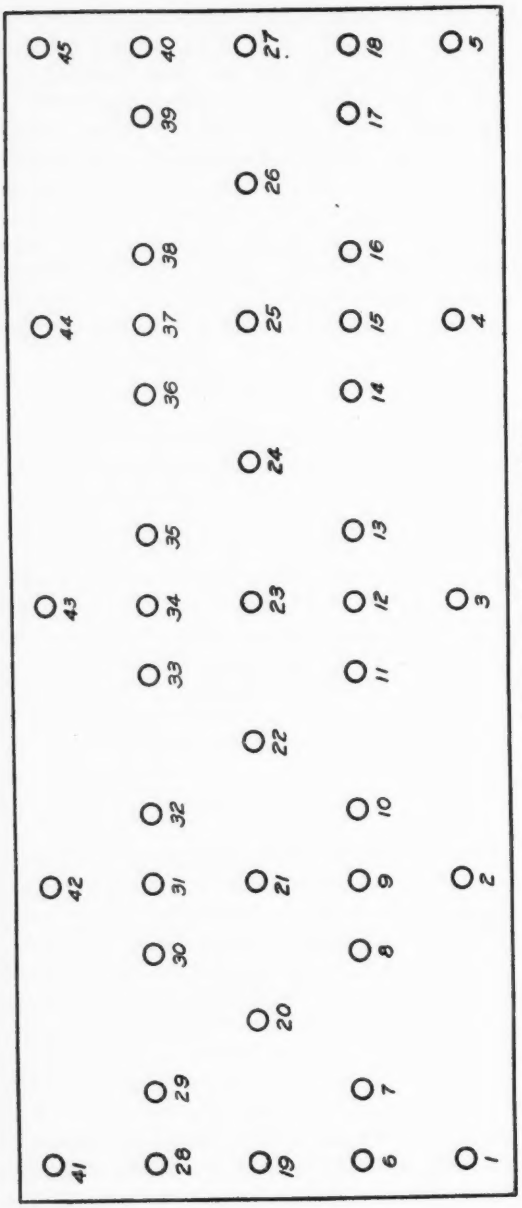


FIG. 1. LOCATION OF BORINGS OVER PLOT

this material was carefully removed with the auger before taking the sub-surface sample. Each boring was placed in a separate container, taken to the laboratory and placed in the drying room. Twenty days later both plots were sampled again in the same manner and within an inch of the same points, the original stakes having never been removed.

Each sample was weighed and prepared for analysis by discarding all material which would not pass a 10-mesh sieve and pulverizing the remainder in a planetary mill to pass a 100-mesh sieve. The weight of the discard was recorded. Nitrogen determinations in 10-gm. duplicates were made of each air-dry sample, making a total for the two samplings of 720 determinations. If the duplicates did not check within 0.1 cc. of $\frac{1}{14}$ *N* alkali they were both discarded and another set was run. This was necessary in only a relatively few cases—the analyst, Mr. J. R. Royston, whose services are acknowledged and appreciated, having had considerable experience in nitrogen determinations in soils.

TABLE 1

Arrangement of borings for composites

COMPOSITE	BORINGS
1	1 to 45, inclusive
2	1, 3, 5, 7, 11, 13, 17, 20, 22, 24, 26, 30, 32, 36, 38, 42, 44
3	2, 4, 8, 10, 14, 16, 20, 22, 24, 26, 29, 33, 35, 39, 41, 43, 45
4	1, 2, 3, 4, 5, 19, 20, 21, 22, 23, 24, 25, 26, 27, 41, 42, 43, 44, 45
5	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40
6	1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 19, 21, 23, 25, 27, 28, 31, 34, 37, 40, 41, 42, 43, 44, 45
7	1, 2, 3, 4, 5, 19, 21, 23, 25, 27, 41, 42, 43, 44, 45
8	1, 2, 3, 4, 5, 20, 22, 24, 25, 26, 41, 42, 43, 44, 45

Plot I was not as uniform in composition as had been anticipated. In the first sampling the nitrogen content of the borings varied from 0.155 to 0.258 per cent in the surface soil and from 0.118 to 0.243 per cent in the sub-surface soil. The nitrogen percentages varied in the second sampling in much the same way, but the highest and the lowest nitrogen content were not always found at the same locations on the plot. In the non-uniform plot the nitrogen content of the individual borings fluctuated from 0.113 to 0.226 per cent and that of the sub-surface from 0.087 to 0.236 per cent, with similar variations in the second sampling. Since individual determinations of both weight of sample and nitrogen content were made, it was possible to calculate what the reported analysis would have been if any given combination of borings had been mixed for a composite sample before analyses were made. The quantity of soil in a boring varied considerably. In the first sampling of plot I the largest core contained 248 gm. and the smallest 116 gm.

Arranging the single borings into eight groups as shown in table 1 according to what might be considered fairly logical schemes of sampling, it was found

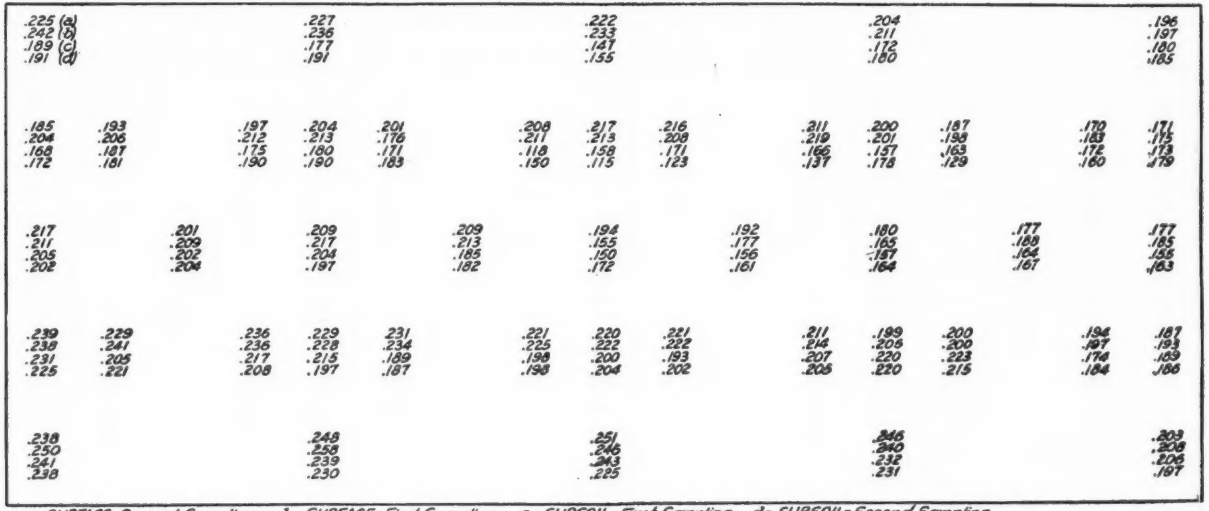
that the analyses which would have been reported for any plot on the basis of these composites varied considerably both with the method of choosing the samples for the composite and the number of samples entering into the composite. Table 2 shows the results of this grouping of samples.

TABLE 2

Nitrogen in composites

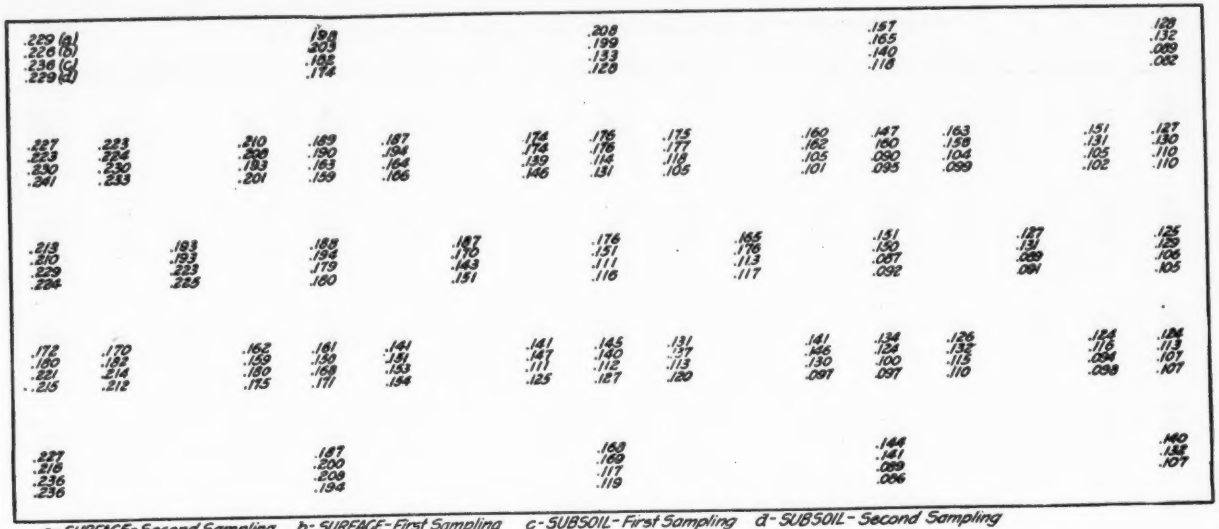
	COMPOSITE	FIRST SAMPLING	SECOND SAMPLING	DIFFERENCE
		per cent	*per cent*	*per cent*
Plot I. 1st depth	1	0.210	0.210	0.000
	2	0.213	0.210	0.003
	3	0.213	0.212	0.001
	4	0.211	0.213	0.002
	5	0.210	0.207	0.003
	6	0.213	0.212	0.001
	7	0.215	0.217	0.002
	8	0.221	0.219	0.002
Plot I. Second depth	1	0.187	0.187	0.000
	2	0.188	0.186	0.002
	3	0.187	0.187	0.000
	4	0.189	0.192	0.003
	5	0.186	0.184	0.002
	6	0.191	0.192	0.001
	7	0.193	0.193	0.000
	8	0.195	0.196	0.001
Plot II. First depth	1	0.166	0.161	0.005
	2	0.168	0.166	0.002
	3	0.168	0.165	0.003
	4	0.173	0.173	0.000
	5	0.161	0.159	0.002
	6	0.168	0.168	0.000
	7	0.174	0.175	0.001
	8	0.175	0.174	0.001
Plot II. Second depth	1	0.144	0.142	0.002
	2	0.144	0.143	0.001
	3	0.146	0.142	0.004
	4	0.148	0.149	0.001
	5	0.141	0.141	0.000
	6	0.147	0.144	0.003
	7	0.150	0.144	0.006
	8	0.150	0.149	0.001

The outstanding feature brought out by this table is that the nitrogen contents of the duplicate composites are quite uniform, no matter how these composites were selected. This indicates that, within certain limits, the number of samples entering into the composite is not so important as that



a - SURFACE - Second Sampling b - SURFACE - First Sampling c - SUBSOIL - First Sampling d - SUBSOIL - Second Sampling

FIG. 2. NITROGEN CONTENT OF SOIL AND SUBSOIL SAMPLES CHOSEN FROM PLOT 1



a- SURFACE- Second Sampling b- SURFACE- First Sampling c- SUBSOIL- First Sampling d- SUBSOIL- Second Sampling

FIG. 3. NITROGEN CONTENT OF SOIL AND SUBSOIL SAMPLES CHOSEN FROM PLOT 2

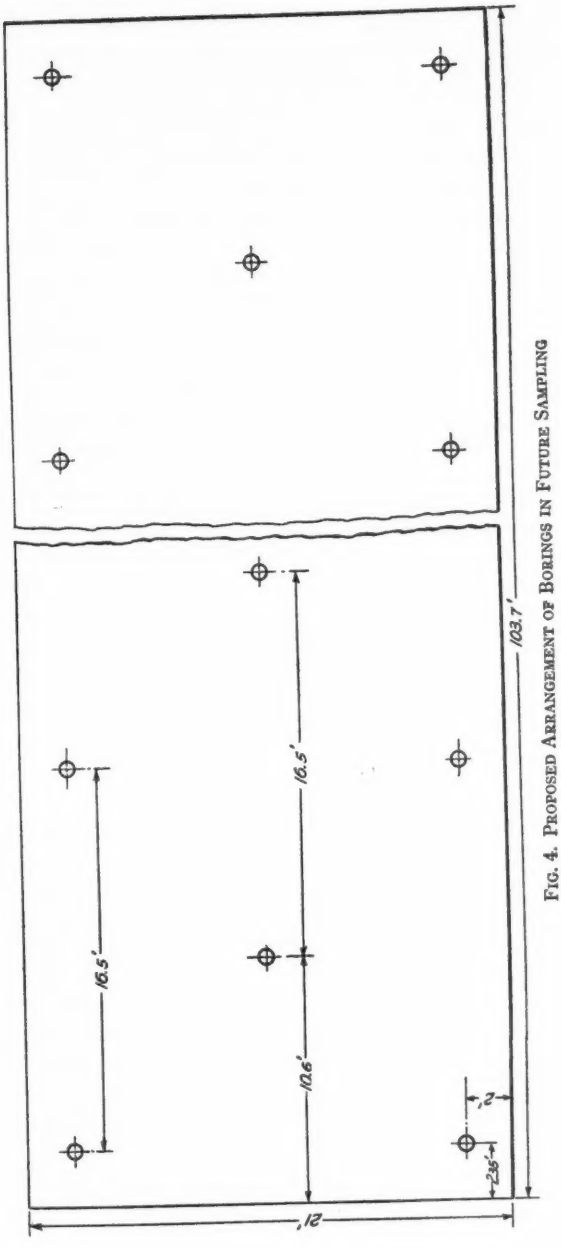


FIG. 4. PROPOSED ARRANGEMENT OF BORINGS IN FUTURE SAMPLING

the samples chosen at different times to represent a plot should be selected from the same locations on the plot. With the more uniform plot the greatest variation was 0.003 per cent, equivalent to 60 pounds of nitrogen per 2,000,000 pounds of soil. With the less uniform plot the greatest variation amounted to 120 pounds per 2,000,000. The composites resulting from the eight groupings of the samples show differences in the nitrogen content of as much as 0.009 per cent, or 180 pounds per 2,000,000.

Reference to figures 2 and 3 shows that it is unsafe to have the composite made up of too few borings. In many cases the cores selected from the same location are almost identical in nitrogen content. At one point in plot 1, however, the variation amounted to 0.039 per cent and at several other points it exceeded 0.01 per cent. It is possible to so select the borings as to show very marked differences in nitrogen content in duplicate composites, provided the number of borings in the composite is not large.

It would appear from a study of these data that selecting the samples according to the arrangement in figures 2 or 3 or a combination of these two could give results very comparable with those secured from taking the entire 45 for the composite. The total number of borings for the composite would then be 17 or in the latter case 30. If the second samples were chosen from the same locations the data would indicate an almost exact duplication of analysis. It would seem logical, however, to arrange these locations uniformly over the plot. On the basis of these analyses it is safe to assume that a composite of 20 borings distributed uniformly over the plot is satisfactory, provided in subsequent sampling the locations of the borings are practically identical with those of the first sampling. The effect of cultural treatment with several years intervening will be left for a subsequent report.

CONCLUSIONS

On the basis of this study it seemed desirable to locate the samples of soil for the composite representing each plot according to the arrangement indicated in figure 4. There appears to be no argument in favor of selecting separate samples of the surface and sub-surface soil, but it does seem desirable that the samples be chosen to a depth well beyond the plow line. According to this plan the composite from each $\frac{1}{20}$-acre plot would be made up of 20 samples, each chosen to a depth of 12 inches and arranged over the plot as indicated in the diagram. In all subsequent sampling of these plots the location of the samples and the method of choosing them should be as nearly an exact duplication of the first sampling as possible.

REFERENCES

(1) BEAR, F. E., AND SALTER, R. M. 1916 The residual effects of fertilizers. W. Va. Agr. Exp. Sta. Bul. 160, p. 13.

(2) HOPKINS, C. G., AND PETTIT, J. H. 1911 Collecting and testing soil samples. Ill. Agr. Exp. Sta. Cir. 150, p. 1–2.

(3) LEATHER, J. W. 1902 Sampling of soils. *In* Jour. Chem. Soc. [London], v. 81, no. 2, p. 883–887.

(4) Official Agricultural Chemists 1916 Recommendations for official and tentative methods of analysis. *In* Jour. Assoc. Off. Agr. Chem., v. 1, no. 4, p. 11–12.

(5) PFEIFFER, T. L., AND BLANCK, E. 1912 Die Bedeutung des Analyzen fehlers bei der Entscheidung von Fragen über den Stickstoffhaushalt des Ackerbodens. *In* Land. Vers. Stat., Bd. 78, p. 367–374.

(6) ROBINSON, G. W., AND LLOYD, W. E. 1915 On the probable error in soil sampling. *In* Jour. Agr. Sci., v. 7, pt. 2, p. 114–153.

(7) THIEL, R. 1905 Über die Schwierigkeit, vermittels der Kjeldahlsehen Methode eine geringe Stickstoffschwankung in Ackerboden festzustellen. *In* Mitt. Landw. Inst. Breslau, Bd. 3, No. 2, p. 157–178.

(8) WARINGTON, ROBERT 1895 The Rothamsted Experiment Station. U. S. Dept. Agr. Off. Exp. Sta., Bul. 8, p. 7–41.

(9) WHITNEY, MILTON 1899 How samples are collected. *In* U. S. Dept. Agr. Bur. Soils Bul. 16, p. 14–15.

(10) WILEY, H. W. 1895 Principles and Practice of Agricultural Analysis, v. 1, p. 61–95. Chemical Publishing Co., Easton, Pa.

THE EFFECT OF AERATION AND OTHER FACTORS ON THE LIME REQUIREMENT OF A MUCK SOIL

SETH S. WALKER

Formerly at the Louisiana Agricultural Experiment Station, Baton Rouge

Received for publication December 30, 1919

INTRODUCTION

After the work reported in this paper had been practically completed the attention of the author was called to a much more elaborate investigation by Conner (1) along the same line. In view of Conner's paper it might almost seem that the present one is superfluous. However, the methods and conditions of the two investigations differed sufficiently to justify publication of the results of both as supplemental to each other.

METHODS USED AND EXPERIMENTAL RESULTS

In April, 1919, the writer had occasion to determine the lime requirement of some muck soil from the shore of Lake Pontchartrain, Louisiana, and was impressed by the fact that the long time required for air-drying, together with the frequent stirring, probably brought about chemical changes which would affect the determination. Such oxidation changes would presumably reduce the acidity. To test this point some of the soil was air-dried in the usual way and ground to pass a 2-mm. sieve. Another portion was kept in the original moist condition in a sealed jar. Moisture was then determined in both the air-dried and the moist portions and the lime requirement of each was determined by the Johnson method (2). The equivalent of 10 gm. of dry soil was weighed in each case and enough water added to the air-dried soil and to the blanks to make the total amount of water the same in all cases. The mixtures of soil and bicarbonate solution and also the blanks were shaken 10 minutes before filtering.

It is recognized that the Johnson method for lime requirement is rather new and has not yet been generally adopted. However, it is rapid, simple, and concise in its results, and is believed to be eminently suited to an investigation like the present one where comparative results only are desired.

Contrary to expectation, the air-dried sample gave a higher result than the undried sample. In other words, it appeared that aeration had increased the acidity.

The question then arose as to whether a drained and cultivated muck soil would actually tend to become more sour than if it were left undisturbed in its original overflowed, water-logged condition. Incidentally, it was desired to know how much effect lime, in the quantity indicated by the Johnson method, would have in reducing acidity through a considerable period of time, and what changes might take place during storage of the moist and air-dried soil, respectively.

TABLE 1

Lime requirement of muck soil after various treatments

(Expressed in per cent of calcium carbonate on the basis of oven-dry soil)

TREATMENT	APRIL		DECEMBER (COMPARE WITH APRIL)	DECEMBER	
	Moisture	Lime requirement	Lime requirement	Moisture	Lime requirement
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
A. None	76.4	0.501, 0.471 → 0.486	0.615	76.1	0.540
B. Air-drying	19.1	0.559, 0.536 → 0.547	0.418	18.3	0.456
C. Aerobic 1				60.7	0.489
C. Aerobic 2				66.6	0.514 → 0.501
D. Anaerobic 1				78.6	0.598
D. Anaerobic 2				83.1	0.617 → 0.608
E. Lime plus aerobic 1				63.7	0.411
E. Lime plus aerobic 2				58.9	0.418 → 0.414
F. Lime plus anaerobic 1				85.0	0.553
F. Lime plus anaerobic 2				85.2	0.534 → 0.543

Different portions of the soil were lettered and preserved as follows:

A. The original moist soil (76.4 per cent moisture), sealed in a glass jar.

B. Same as A, only air-dried and ground to pass a 2-mm. sieve. Kept in an ordinary screw-top sample jar.

C. Same soil as A; 150 gm. in each of two glass jars, 1 and 2. Jars kept uncovered. Soil kept moistened with distilled water and stirred occasionally.

D. Same soil as A; 150 gm. in each of two glass jars, 1 and 2. Soil covered with distilled water and jars kept tightly sealed.

E. Same as C except for addition of calcium carbonate in the amount indicated by the original determination on A.

F. Same as D except for the addition of calcium carbonate as in E.

The tests were started in April, 1919, and continued approximately 8 months. At the end of the period (December, 1919) the contents of jars D and F were transferred to large porcelain dishes and exposed to the air, with occasional stirring, for a few days until the excess of water had evaporated. Moisture was then determined in all of the samples, including the original A and B, and the lime requirement was determined by the Johnson method, distilled water being added to all samples (except the wettest one—F2) and to the blanks in quantity to make the relation of dry soil to total liquid the same in all cases.

Also, determinations were made on A and B using the same quantity of dry soil and total liquid as was used for the original determinations on these samples in April, in order to afford direct comparison between the two dates.

The results obtained, expressed in percentage of calcium carbonate required to neutralize the oven-dry soil, are shown in table 1.

DISCUSSION OF RESULTS

The figures given in table 1 plainly indicate that more acidity was developed under water-soaked, anaerobic conditions than when the moist soil was kept aerated by stirring. Comparing C with D and E with F it will be seen that the lime requirement of the anaerobic soil exceeds that of the aerobic soil by more than 0.1 per cent in each case. However, in view of Conner's results (1) showing that the acidity developed by a peat soil is greater the more water it contains, it cannot be considered as proved that aeration was the controlling factor in the experiments here reported. D and F were much wetter than C and E, respectively, and therefore would probably have developed more acidity even if C and E had not been mechanically aerated. But of course the logical interpretation of Conner's results, as he himself recognizes, is that the drier soils are better aerated by diffusion than are the wetter soils, and that the oxidation thus accomplished is at least one factor which tends to decrease acidity.

If the Johnson method furnishes a true or even approximate measure of soil acidity, then it appears that the addition of lime to this soil has greatly increased the rate of formation of acids. Thus sample E, which was supposed to be exactly neutral at the beginning of the experiment, developed during the 8 months a lime requirement of 0.414 per cent, while sample C, which started with a requirement of 0.486 per cent, showed 0.501 per cent at the finish—or a difference of only 0.015 per cent developed during the 8-month period. Similarly, sample F, which was neutralized with lime at the beginning, developed an acidity of 0.543 per cent, while sample D, without lime, developed only the difference between 0.608 and 0.486 per cent, or 0.122 per cent. This accelerated rate of acid formation is probably not to be explained by the presence of the calcium, but by the Law of Mass Action. The exactly neutralized soil would naturally develop acidity much more rapidly than one already containing much acid.

However this may be, it is evident that in liming a muck soil for permanent results one should apply much more lime than is indicated by the lime-requirement determination, in order to take care of subsequently developed acidity.

As already noted in the first part of the paper, sample B, air-dried, showed a higher lime requirement than sample A, undried. Conner also found that air-drying increased the acidity in many cases, though not in the case of an organic soil containing much water, such as sample A.

But when samples A and B were kept in storage for 8 months some notable changes took place. Sample A, containing 76 per cent of water, developed a considerable increase of acidity (changing from 0.486 to 0.615 per cent) while the air-dried sample (19 per cent moisture) changed in the other direction, from 0.547 to 0.418 per cent.

Some light is thrown on the nature of the Johnson method for lime requirement, and all similar methods, by a consideration of the two sets of values found for A and B in December. In the first case, where the same ratio of dry soil to total liquid was used as had been used in the April determinations, somewhat different results were obtained than in the second case where a greater amount of total liquid was used in order to make the results comparable with those of C, D, E and F. This, of course, merely emphasizes the well known fact that all such methods are only comparative, not absolute, and that conditions must be rigidly controlled in using them.

SUMMARY

A typical black muck soil was treated in various ways in order to study the effect of aeration and other factors on lime requirement as determined by the Johnson method.

It was found:

That the lime requirement of this soil was increased by air-drying.

That when the soil was kept moist and frequently stirred for a period of 8 months the increase of acidity was decidedly less than when it was kept covered with water in a sealed jar.

That soil which was first neutralized with calcium carbonate and then kept for a period of 8 months, under either aerobic or anaerobic conditions, developed a great deal more acidity than did unneutralized soil under the same conditions. This is explained by the Law of Mass Action.

That the lime requirement of all samples stored in a moist condition was increased, but that the air-dried sample decreased in acidity during storage.

It was emphasized that results obtained by the Johnson and similar methods are comparative rather than absolute, and that rigid conditions must be adhered to in using such methods.

REFERENCES

(1) CONNER, S. D. 1918 Soil acidity as affected by moisture conditions in the soil. *In* Jour. Agr. Res., v. 15, no. 6, p. 321.

(2) JOHNSON, M. O. 1918 Reaction of Hawaiian soils with calcium bicarbonate solutions, its relation to the determination of lime requirements of soils, and a rapid approximate method for the determination of lime requirements of soils. *In* Jour. Indus. Engin. Chem., v. 10, no. 1, p. 31.